X. *Contributions to our Knowledge of the Fucaceæ: their Life-History and Cytology.**

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[Plates 19–24.]

Development of the Oogonia and Oospheres.

The following account of the oogenesis refers especially to *Fucus vesiculosus*, *F. serratus*, and *Ascophyllum nodosum*, except where the contrary is stated :—

The first sign of the young oogonia consists in the projection of the cells forming the wall of the conceptacle into its cavity. The protoplasm of the papilla in well-fixed material appears finely granular, and in very thin sections it can be seen that this peculiarity is due to a reticulate or alveolar structure, the latter explanation being by far the most probable one. The nucleus is large, and is often excentrically situated in the cell. It is difficult to procure good material for the study of the division of this nucleus, but it is most important to be sure of the chief processes connected with its karyokinesis. It is best to collect the plants shortly after they have been covered by the flowing tide; indeed, we found it to be almost impossible to secure the required stages at any other time. Our own practice has been to take a boat, and to gather and fix the material on the spot, cutting up the receptacles of the fertile thalli, and dropping the pieces at once into the fixing solution. As the result of a lengthy series of trials we found the best material was obtained by using either Flemming's (strong) or Hermann's solutions with half the normal amount of osmic acid. We obtained very good results by making up these fixatives in 50 per cent. sea-water.

When the projecting papillæ are about to enter on their first stage of division the nucleolus is seen to become vacuolated, although it does not disappear till just before the grouping of the chromosomes in the equatorial plane, at which stage we could no longer distinguish it. But the fact must not be lost sight of that it loses part of its contents at a very early period of the karyokinetic process.

* *Cf.* our former paper "On Fertilisation and the Segmentation of the Spore in *Fucus*," 'Roy. Soc. Proc.,' vol. 60 (1896).

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A fine chromatic reticulum is formed, and beautiful centrospheres were always seen at the same time situated at opposite points of the nucleus. We were, however, unable to ascertain the exact mode of origin of these structures. Often also granular inclusions could be detected within the centrospheres, and they certainly represent the bodies which have been described as centrosomes. But their variability, both in size and number, within even one pair of centrospheres, is so great that we do not feel disposed to attach much weight to them as morphological cell elements, especially as they could not be recognised in a number of instances in which the cytoplasm was very finely fixed.

The spindle formation was not studied in any detail in this mitosis, because sufficient material was not available for an exhaustive examination of this point; but what we did make out led us to conclude that the process here is not essentially different from the later divisions, of which we had a large number of examples. The chief difference lay in the relatively great breadth of the spindle in this first division, recalling that characteristic of the thallus cells of the *Fucus* plant, and it is clearly related to the large number of chromosomes, which we estimated at about 26–30. It is extremely difficult to be quite certain as to the exact number; countings of a number of cases gave an average of 28 in the three species we especially studied.

Strasburger, however, considers 30 to be the probable number in the form (*F. platycarpus*) which he especially studied. His figure* for this plant, compared with our own (Plate 19, fig. 1) for *Ascophyllum*, shows how uniform is the general character of the mitosis in the various related species of Fucaceæ, for the corresponding stage in *F. vesiculosus*, in which we have also seen it, shows no essential points of difference. During, or soon after, the reconstitution of the two daughter nuclei one of them moves to the base of the cell, whilst the other remains in the projecting papilla, and a wall is then formed cutting off the true oogonium from the stalk cell.

The oogonium next increases greatly in size, and its cytoplasm is granular and betrays clearly a foam structure in well-fixed material. The nucleus is of a large size. As the division of the nucleus draws near, the cytoplasm undergoes a change which in plants that have been preserved with any preparation containing osmic acid is very marked owing to the fact that the acid is very slightly or not at all reduced at this stage. Thus the oogonia in this condition are easily distinguished from the rest, even under a magnifying power far too low to admit of structural details being discerned at all. This fact is of interest, indicating as it does that special metabolic processes are going on within the cytoplasm, and that they are associated with karyokinetic activity.

The nuclei are, as has been said, of a large size, and they contain one or more nucleoli, together with a beautifully clear chromatic network, which is even at this stage seen to be markedly polarised (figs. 2, 3). Commonly, at the same time, a

* Pringsheim's 'Jahrb. f. Wiss. Bot.,' vol. 30, Plate 17, fig. 3A.

single centrosphere can be seen at one side of the nucleus, though it is not always definitely related to the polarisation of the chromatic thread. Later on a second centrosphere becomes visible; and from the fact that the two are not always situated at 180° apart from each other, it might be inferred that they have had a common origin; but we have never been able to satisfy ourselves as to this, either in this or in the other divisions. Indeed, in some cases it seemed almost certain that the second one was formed independently of the first, and was differentiated *ab initio* at some distance from it.

The chromatic thread is now seen to be double in many places, indicating a longitudinal fission; but the object is too small, and the various steps are gone through too rapidly to permit of this point being investigated by us in greater detail. We are, however, quite satisfied that the longitudinal fission exists at this stage, just as it does in the corresponding mitosis in higher plants and in animals.

As soon as the chromatic thread breaks up into its chromosome elements, it can be readily seen that a reduction in their number has occurred since the last nuclear division. In our previous communication we regarded it as almost certain that the reduction occurred at this point, and STRASBURGER* has since shown that it does so in the species of *Fucus* which he investigated. In *Ascophyllum*, the plant in which we have been best able to trace the process, it is extremely obvious that a change has occurred. Not only the character of the spindle, but also the form of the chromosomes, differs totally from the corresponding structures in the previous mitosis. The spindle is slender and proportionally elongated, whilst the chromosomes, notwithstanding that their small size renders it very difficult to follow their changes of form in great detail, by their early longitudinal fission and their humped appearance when on the spindle, lend support to the view that this mitosis is quite comparable with the peculiar heterotype division connected with the reduction phenomenon in higher plants and animals.

An interesting feature presented by the achromatic spindle in this and especially also in the following oogonial divisions, as well as in the divisions of the oospore, lies in the fact that it is largely intranuclear. It begins to be formed *before* the nuclear wall can be seen to be broken down at the two ends, and this points to a mixed origin for the spindle itself; for whilst the polar radiations from the centrospheres are certainly cytoplasmic, it seems highly probable that a part, at any rate, of the interpolar part of the structure is of nuclear origin. Later on the nuclear wall breaks down or thins out at the poles, although the lateral walls still sharply delimit the nuclear space from the surrounding cytoplasm. The polar radiations from the ends of the spindle for the most part end in the protoplasmic substance of the oogonium, and do not reach to the outer limit of the cell. Some importance has been attached

* STRASBURGER, "Kerntheilung u. Befruchtung bei *Fucus*," Pringsheim's Jahrb. für Wiss. Bot., vol. 30 (1897).

to this fact by DRÜNER,* in opposition to HEIDENHAIN; but our own studies have convinced us that both kinds of radiations exist, sometimes the achromatic fibres appearing to end on the wall, whilst in other instances none could be traced so far, but all ended in the internal cell-protoplasm. The persistence of the radiations throughout the equatorial plate stage is worth noting, for in many of the higher plants this is not the case; and in *Pellia*, a liverwort in which they are very prominent during the early and late phases of mitosis, they completely disappear in this intervening stage. Not improbably the fact is to be correlated with the rapidity with which the karyokinetic process is passed through in *Fucus* as compared with many other organisms. We have already hinted at evidence pointing to this conclusion in our remarks on the importance of securing the plants during a certain state of the tide. The appearances of the mitotic phenomena are such as to bear out the conclusion, arrived at on other grounds, that in *Fucus* karyokinesis is associated with external conditions, and that these periodically-recurring conditions only persist for a short time. No walls are produced in connection with either this or the next nuclear division in the oogonium.

The second karyokinesis follows quickly upon the first, and after it is over the four nuclei are seen to lie close together in the middle of the oogonial cytoplasm (fig. 7). They are invested in a peculiarly differentiated mass of protoplasm which in the fresh state is of an olive colour, and after fixing and sectioning it is seen to be much denser than the rest of the cytoplasm. STRASBURGER has figured this aggregated appearance in the case of *F. serratus*, and we have observed it in all the species examined by us, and also in *Ascophyllum*, though it is not nearly so prominent in the last-named plant.

A considerable interval of rest seems to obtain between the second and third (final) oogonial mitosis. The first indication of its approach is seen in the differentiation of centrospheres in relation to each nucleus. The nuclei become drawn out into an oval or pointed form (figs. 9–15), and simultaneously move away from each other. The conclusion is irresistibly borne in on the mind of the observer that this separation is due to the mutual repulsion of the centrospheres. The nuclei eventually lie with their elongating axes in different directions, so that it is often possible to obtain polar and profile views of different ones in the same section. The centrospheres are amongst the finest that can be imagined, and the great contrast between the area they occupy and the rest of the protoplasm renders it easy to define almost sharply the neutral zones between them.

The chromatin is chiefly discoverable near the two ends of the nuclei, the equatorial region being at first occupied by a large nucleolus. This latter early exhibits signs of vacuolation, as though some portion of its substance were passing out from it in solution. The linin framework rapidly assumes a more regular appearance, and the

* DRÜNER, "Studien ü. d. mechanismus d. Zelltheilung," Jenaische Zeitschr., vol. 29.

chromatin becomes clearly disposed in lines or bands. When the chromosomes are fully formed, they are seen, as in the previous division, to be present in the reduced number (about 14 or 15). The spindle has meanwhile been formed between the poles, and it seems quite certain that it is, in part at least, of nuclear origin. In favourable cases lines could be detected before there was any conclusive evidence of the possibility of cytoplasmic irruption, and we have multiplied the instances in which (as described in our previous paper) we have succeeded in contracting the whole spindle away from the nuclear wall at one end, leaving it as a sharply-ending cone in the still persisting nuclear cavity.

The diaster commences rather irregularly, and it is not always easy to identify the individual chromosomes, but we have been able to fully convince ourselves that the process differs in no essential particulars from that occurring in the higher plants.

When the daughter nuclei are fully formed the connecting achromatic fibres disappear, and the eight nuclei lie free in the oogonial cytoplasm.

It is of special interest to notice that in *Ascophyllum* this division, leading to the production of eight nuclei, occurs (fig. 14) as in the species of *Fucus*, although only four of them are destined to become associated with the formation of eggs. We endeavoured to ascertain whether the four survivors were each derived from one of the four preceding nuclei, or whether their destiny was fixed during an earlier division, but we were not successful in determining this point. However, our observation on the karyokinesis conclusively proves the correctness of OLTMANNS' statement* as to the degeneration of four superfluous nuclei during the differentiation of the oospheres of this plant. The degeneration occurs very soon after the formation of the eight nuclei, and therefore is easily overlooked.

The oogonial wall, in the plants studied by us, is at this time undergoing a differentiation into two layers (figs. 16–18), which soon become separated from each other through almost their whole extent. The outer membrane remains comparatively thin, whilst the inner one, at first fairly thin also, becomes subsequently much thicker; this is probably to be accounted for partly by a change in the wall itself, resulting in its stratification being rendered more apparent, and partly by actual additional thickening. At the base of the oogonium the two membranes remain united, and in the region of the well-known basal pit the common membrane is very thin.

When the oogonium is about to undergo septation, the nuclei again become associated with a change of appearance in the protoplasm. The latter is for the most part densely granular, but in the vicinity of the nuclei the granules are of very small size, producing the effect of turbidity, and the chromatophores do not occur in these regions. As the turbid spheres surrounding each nucleus enlarge, the coarser cell inclusions become driven farther and farther from each nucleus, and

* OLTMANNS, "Beiträge z. Kenntnis d. Fucaceen," Bibliotheca Botanica, Heft 14 (1894).

finally are heaped up round the periphery of the protoplasm, and especially in the neutral planes which delimit the different oospheres. It is in these neutral planes that the cell walls arise. The protoplasm forming them stains differently from that which fills the greater part of the rest of the oogonium, a fact noted also by STRASBURGER. We find in preparations stained with dyes which colour cellulose, that they are especially absorbed and most persistently retained in these planes, and also by the granular protoplasm near the periphery of the oogonial contents as a whole. Ultimately membranes are formed which divide the oogonium into as many chambers as there will be oospheres produced. We notice that the membranes are formed first in the protoplasm, and do not become attached simultaneously to the oogonial wall, though this happens very shortly after their appearance. We were thus led to enquire whether each egg may not secrete a wall all round itself, the peripheral portion being, however, in close contact with the oogonial wall. But after a careful study of a large number of preparations we have come to the conclusion that a peripheral membrane independent of the oogonial wall is not formed at this stage, although later on the oospheres are found to be enclosed in a common membrane which becomes detached from the rest of the oogonium. Of course, this opinion does not affect the possibility of a continued thickening going on in the oogonial wall itself, to which the partitioning membranes are attached, and indeed we regard this as almost certainly taking place, basing our opinion mainly on the similarity of the staining reaction of the protoplasm which lines the whole surface of each egg at the time when the intervening membranes are undoubtedly increasing in thickness, and also on the alteration which the oogonial wall itself exhibits. An inner shell, which is directly continuous with the septa, separates off from an outer one, the two only remaining in contact at the base of the oogonium in the region of the basal pit already spoken of. Thus at this stage the eggs are enclosed, 1st, in a common wall on to which the partition walls directly abut; 2nd, by a second middle layer still consisting of cellulose, and attached to the inner one at the base; and 3rd, by the outermost membrane, which remains thin, and which is also attached to the rest at the base. It will be convenient to distinguish these three membranes as *Exochite*,* *Mesochite*, and *Endochite* respectively.†

In some cases the partition walls between the individual eggs become double (fig. 17*b*) by internal differentiations. When this occurs they are seen to be continuous with the endochite, which subsequently may break up, so that each egg becomes isolated, and then is seen to be surrounded by a cell-wall of its own. Though these cases are not frequent they serve to strengthen our view as to the probable connection between the formation of the endochite, or at least of its inner layers, with the protoplasm of the segregating oospheres.

* Χιτών is the word used in classical Greek to designate the coats of an onion.

† The Mesochite and Endochite are respectively equivalent to the *perispore* and *episporc* of DECAISNE.

Liberation of Sexual Products.

When plants of *Fucus*, *Ascophyllum*, or *Pelvetia*, bearing mature receptacles, are exposed during the ebb tide under normally favourable conditions, mounds of mucilage containing sexual products are found over the openings of the conceptacles.

This phenomenon has often been described, and, amongst other observers, Thuret* and Oltmanns† have pointed out the erroneous character of the suggestion sometimes put forward, namely, that this is the only mode of liberation of the sexual cells in these plants. A number of often-repeated observations has convinced us that exposure to the air is not only unnecessary, but that in some cases its effects may be directly injurious. Thus if mature plants are kept in a room for more than a day in a moist atmosphere, it is found that a large number of quite immature oogonia are extruded, often before the oospheres have been themselves differentiated. In *Pelvetia* the entire oogonium together with its stalk cell is frequently, under these conditions, torn off and expelled, and in many instances the products normally extruded between tides are found to consist partly of immature sexual cells. Such observations prove that some sort of mechanical pressure is exerted on the contents of the conceptacles, and it is produced, at least in part, by a shrinkage of the cortical cells of the plant which results in their sinking inwards and thereby exerting a pressure upon the mucilaginous cells of the medulla, and hence of course also upon the inner wall of the conceptacles. Furthermore, this contraction of the cortex causes the ostioles to gape widely, thus providing ready facility for the egress of the conceptacular contents.

The production of a supply of free mucilage is essential to the ensuring of proper extrusion. Young, and consequently immature, receptacular portions of the frond of *F. platycarpus* for example are found when cut open to present a woolly appearance in the interior. This is due to the fact that the loose medullary cell-rows have not undergone that change in their walls which, later on, leads to the production of a plentiful supply of mucilage. The latter, however, becomes extremely abundant when the period of maturity arrives.

On the other hand, the tissues of old receptacles are found to be destitute of mucilage (which has all been long ago squeezed out); and although examination of the conceptacles may shew them to be full of ripe sexual cells, no extrusion takes place under these circumstances.

Again, if receptacles which have begun to extrude are placed in sea-water, it can be seen that the expelled products are pushed away from behind as the additional ones are squeezed out from the interior of the conceptacles, and that the pressure thus exerted is considerable is shewn by the fact that, in *Halidrys* especially, small pieces of the eggs may be pinched off at the ostiole. The sterile conceptacular hairs

* Ann. Sci. Nat. (Bot.), 4th Ser., vol. 2 (1854), p. 200.

† *Loc. cit.*

also doubtless share in the process of expulsion; for if sections of *Pelvetia* be examined in water, these hairs are seen to swell up, and even almost to resemble the oogonia save in their poverty as regards protoplasmic contents. Anyway, it is probable that these paraphyses may serve to guide the loosened sexual cells towards the ostiole. The mucilage is not only derived from the medullary cells of the plants, but also from the swelling oogonial walls; and this last is a factor of importance, since it is only operative at the time of actual maturity of the sexual products. The whole process, however, is clearly related to the habits and needs of the particular plants, and the variations exhibited between the different individuals are markedly correlated with their special requirements. Thus the extrusion, even in forms which are never exposed, does not go on continuously, but recurs at intervals. Mature conceptacles of *Halidrys*, for example, when placed in a vessel of sea-water, only expel their sexual cells at intervals of many hours; and it is possible, after keeping the plants for several days, to find groups of spores in advanced stages of germination, whilst clumps of oospheres may also be seen surrounded with moving antherozoids, proving that in them fertilisation has not yet taken place.

The changes which result in the liberation of the individual eggs in the water vary somewhat in different genera, and are not always constant, even within the same species. We have already referred to the oogonial wall as being differentiated into exo-, meso-, and endochite. The exochite remains attached to the stalk cell during the extrusion of the sexual cells, whilst, as a rule, the mesochite and endochite come away with the oospheres. They may be, perhaps, appropriately termed the "egg-sac"; that is, membranes which enclose the eggs. Both the endo- and mesochite commonly cohere for a while by the basal pit portions of the membrane; but the inner layers of the mesochite soon become mucilaginous, especially near the apex, and thus a turgid space is formed between the outer and inner of these egg-sac membranes. The mesochite eventually ruptures at the apex, owing to the more rapid gelatinisation of an area of this region of the membrane. Finally, the endochite, together with the partition walls between the individual oospheres, also disappears, by dissolving in the water as mucilage, and the eggs are thereby set free in the water. Sometimes the endochite and partition walls have already become unrecognisable, even before extrusion from the conceptacle has been accomplished.

A less usual variant occurs in *F. vesiculosus* and *F. serratus*, and in *Ascophyllum* when the partition walls, instead of swelling up and disappearing, split so as to form, together with portions of the endochite, coverings for the individual eggs. And it may happen that the membrane thus remaining round each egg does not disappear at all, however long they may be kept in the sea-water. This fact has its bearing on THURET'S experiments on unfertilised eggs to be referred to later.

As regards *Ascophyllum*, THURET has figured ("*Ét. Phyc.*," XXII., 12) a case which frequently occurs in this species, although, as far as our material is concerned, not the most common one. We find that in the majority of instances there is no sharply-

defined membrane present, but that a general swelling of the mesochite is more usual. At the beginning of the swelling a beautiful and delicate stratification is visible, and if stained with methylene blue a number of fine secondary pits become visible in it. It will be remembered that in some other forms, especially in *Pelvetia*, secondary pits are found (see THURET's figs. 12, 13, 14, *ibid.*), and that it has been conjectured that in the latter plant they had some part to play in permitting fertilisation to take place. This hypothesis is not, however, strengthened by their occurrence in *Ascophyllum*, in which plant they unquestionably stand in no direct relation to the process of fertilisation.

FERTILISATION OF THE OOSPHERES.

We have already* described our methods for obtaining a plentiful supply of oospheres for fertilisation stages. Our investigations were chiefly restricted to dicecious species, for the obvious reason that it was possible to control the observations more easily.

When vigorous antherozoids are transferred to vessels containing healthy oospheres they at once congregate around them, and attaching themselves to the periphery of the eggs, cause the well-known movements by lashing the water with the free cilium. But, as THURET noticed, fertilisation can often be effected without any whirling movements taking place, and we have observed perfectly normal germination to follow on the addition of apparently inactive antherozoids to the oospheres.

As regards the actual manner of entrance into the egg on the part of the antherozoid, and of its fusion with the nucleus of the latter, we have but little to add to our previous account. The passage through the cytoplasm is extremely rapid, as is proved by the rarity of specimens which we have obtained which show the antherozoid between the egg periphery and the centrally placed nucleus. In its path through the cytoplasm it rather resembles a chromatophore, from which, however, its reaction to stains readily serve to distinguish it. It travels towards its destination with its blunter end forward (fig. 19), and no trace of cilia could be discerned when it was once inside the egg. We have in our earlier paper commented on the absence of a recognisable centrosome in connection with it, and STRASBURGER also was unable to identify any such structure in those cases which he examined. Nor is there any trace of special protoplasmic differentiation, such as an archeplasm, to be detected. The cause of its rapid movement through the cytoplasm is obscure, but taking its shape into consideration it seems as if it were guided and pushed on by the egg cytoplasm. Arrived at the periphery of the egg nucleus the sperm increases somewhat in size, and rapidly becomes flattened out upon the nuclear periphery, causing an appearance of a swelling on this side. At

* Roy. Soc. Proc., *loc. cit.*

the same time it becomes larger and stains more diffusely, owing to the less condensed state of its chromatin. At first about the size of the egg nucleolus it becomes much larger, and finally it is no longer recognisable. Stages in the fusion of the two nuclei are shown in figs. 14–24 and 47–49. After fusion has taken place a second nucleolus often appears within the fusion-nucleus, and STRASBURGER is probably correct in deriving it from the antherozoid. Not that it is brought in *as such* in the latter body, but that it becomes differentiated after entrance of the sperm into the oosphere, at the expense of some other constituent of (probably) the sperm-nucleus.

There exists a great deal of difference between the degree of attraction exerted on the antherozoids by the oospheres under different conditions. Thus at times, especially when the extruded products have been long exposed to a moist atmosphere so that all the membranes have become deliquescent, the antherozoids are hardly influenced by the presence of the oospheres. On the other hand the oospheres which still retain their walls become covered with the antherozoids. The act of fertilisation occurs in the case of normal healthy products within a very few minutes after the addition of the male cells, and eggs thus fertilised at once form a membrane around them and behave in a very different manner from those which have not become fertilised. Thus, if the sea-water be gradually drawn off from a dish containing a mixture of fertilised and unfertilised eggs, such as may easily be secured by performing the experiment three or four minutes after adding the antherozoids, those which have not been fertilised flatten out, and then the cytoplasm and granular contents lose their coherence and become distributed in all directions. The others, on the other hand, only show local protuberances or only burst at one point. Ten minutes after mixing the sexual cells all the eggs are found in the latter condition.

A successful method of demonstrating the existence of a wall around the protoplasm of newly fertilised eggs consists in replacing the salt by fresh-water. They then burst at one point, and part of the contents is ejected through the hole, often forming moniliform processes as in the reputed germination-attempts mentioned by THURET. Unfertilised eggs similarly treated do not burst, but merely swell and alter their appearance.

In most cases only one antherozoid succeeds in penetrating the egg, but we have seen, amongst several thousand preparations, three cases of polyspermy (fig. 23) in which two antherozoids had effected an entrance. This rare occurrence of polyspermy under the conditions such as are normal for the plants concerned, makes one feel rather doubtful as regards those cases of polyspermy reported for animals; not that the facts are disputed, but as to whether the occurrence in those recorded cases may not be largely the result of the prevalence of abnormal conditions at the time of fertilisation.

We have never succeeded in obtaining satisfactory evidence to show that unferti-

lised eggs are ever able to germinate. THURET, however, mentions that unfertilised eggs which he kept for several days produced moniliform projections, which he regarded as attempts at germination, and he further states that cellulose walls are sometimes present.* As far as our experience goes we have never been able to observe any cellulose walls produced around unfertilised oospheres, but one often observes, on testing the oospheres as soon as they are liberated, that amongst them there often occur egg-like bodies provided with walls. These, however, are either undivided oogonia or else oospheres which have retained their special membrane, instead of its having dissolved away in the water. Again, we find in culture material consisting entirely of normal healthy eggs, that if it be left too long the external protoplasm becomes viscous, and then drawing off the water causes them to be drawn out, instead of effecting their disintegration. But no cell wall could be detected. Changing the dense (evaporated) sea-water, and replacing it by that of normal density, always causes rupture of the immature oogonia or abnormal eggs, and in oospheres, in which the outer layers have become viscous, moniliform processes may be extruded. This latter effect is only seen in eggs which are still living. A remarkable confirmation of the view that the entrance and influence of the male cell forms the stimulus which leads to the formation of the cell wall is shown by the behaviour of oospheres of *Halidrys*. It has already been remarked that during extrusion, pieces of the oospheres are sometimes nipped off during their passage through the ostiole, and that these attract the antherozoids just like normal nucleated eggs. It sometimes happens that these non-nucleated fragments become fertilised, and then they are seen to surround themselves with a cell wall in the normal manner, whilst this never occurs, even in uninjured eggs, if they have escaped fertilisation.

Halidrys offers some features of great interest with regard to its fertilisation, and we shall therefore briefly describe the process here.

On watching the behaviour of the antherozoids when swimming amongst the oospheres, they are seen to attach themselves to the surface of the eggs by one cilium, whilst they maintain a circular or gyratory movement around their point of attachment. Most often there are a number—a dozen or more—of these groups, each consisting of 4–12 antherozoids, distributed over the surface of each oosphere. The movement is always in the clockwise direction, and the chromatophore is on the end of the antherozoid remote from the egg. The rate of gyration is fairly rapid, 40–50 complete turns being made in a minute.

After this has been going on for a while the egg itself evinces a change, swelling somewhat and appearing more transparent than before. Sometimes movements of vacuoles may be discerned, and even the position of the nucleus may change.

* THURET, Ann. des Sc. Nat., 4th Ser., vol. 2, p. 205; also, "Deuxième Note sur la Fécondation des Fucacées," Mém. de la Soc. des Sc. Nat. de Cherbourg, 1858.

These alterations ensue as the definite result of the stimulus in some way given by the antherozoids themselves.

Suddenly the antherozoids are seen to leave the egg like a crowd of startled birds, or else they become quiescent, and these phenomena are immediately followed by a great change in the egg itself, which becomes warty and covered with conical projections. From each papilla a fine thread projects, consisting of a moniliform series of droplets, and antherozoids may sometimes be observed attached to these threads. After the lapse of a few (3–5) minutes the egg resumes its spherical form whilst at the same time its diameter becomes smaller. Still later the fine threads also disappear, whilst the egg regains its original size. As long as the antherozoids are in active motion on the surface of the egg, the latter exhibits a scarcely perceptible rocking movement, and is free in the water, but during the events which have just been narrated it becomes attached to the surface on which it may be resting. We consider it as certain that the flight of the supernumerary antherozoids marks the moment of actual fertilisation, and it seems only possible to interpret the events outside the egg as the result of an excretion from it of some substance which not only exerts on the surrounding antherozoids a negative chemiotactic, but also a directly injurious effect, for a number of dead sperms may be seen around the fertilised egg. Possibly the bead-like filaments, which partly stain like mucilage, are directly concerned in the process.

Eggs which have exhibited the above-mentioned peculiarities at once become invested in cell walls, and after a short period of apparent rest enter on active cell-division. On the other hand, oospheres which have not passed through these stages produce no cell walls, and after a time disintegrate.

Frequently, as already stated, the oospheres lose all their membranes, owing to their gelification and solution, whilst they are still in the conceptacle, and as they pass out of the ostiole often become fragmented. The fragments vary very much in size, and may be as small as the bulk of four or five antherozoids. Nevertheless they attract the male cells quite as effectively as the full-sized nucleated eggs, and they exhibit the same phenomena of swelling and protrusion of papillæ and filaments, and finally they form an enveloping cell wall exactly as do the uninjured nucleated oospheres, but they never divide.

The time taken to effect fertilisation is much longer in *Halidrys* than in *Fucus*, varying from 15–60 minutes, and the small fragments require as long as the entire egg. This shows that at any rate mere size is not the explanation of the delay. The movements of the antherozoids become much more rapid than before, as the moment of fertilisation draws near, and they may now exhibit as many as 60–70 gyrations per minute. At times it happens that papillæ are formed at one end of the egg before they appear at the other. When this is the case, the antherozoids exhibit the accelerated movement at that end at which the protuberances are first formed, whilst those at the other end are gyrating at ordinary speed. Then those at the former end

cease moving or rush away, whilst those at the latter are still rotating. As the change passes over the whole egg, however, all the antherozoids either move off or become quiescent.

As regards the large oosphere-like bodies in *Fucus* with two (or more) nuclei, which have been taken by BEHRENS* for fertilisation stages, we have referred to them in our previous paper, and they require no further discussion here beyond a mere reiteration of the fact that they certainly represent either abnormally developed oospheres or oogonia. (See figs. 45 and 46.)

After fertilisation of the egg of *Fucus* or of *Ascophyllum* has taken place, a remarkable change comes over the structure of the protoplasm. Whereas before this event the cyptoplasm exhibited a somewhat frothy character, and its chromatophores and other bodies of an undetermined nature (physodes, granules, &c.) were irregularly distributed through it, the foam now assumes a more orderly appearance. The walls of the spaces being orientated with a distinct reference to the centrally placed nucleus, the general impression conveyed is that of a radially arranged structure, and this is specially due to the fact that the large chromatophores, which lie in the foam walls, or at the angles where foam cavities abut on each other, for the most part come to lie with their longer axes directed from the nucleus towards the periphery of the egg. The alveolar structure of the protoplasm is very well shown at this stage, although it is, perhaps, not identical with the structure described by BÜTSCHLI† for protoplasm. We say this because the cavities are so much larger than were contemplated by the writer just mentioned, who expressly states that these coarse foams are not to be confounded with those which he was considering. But whether this be so or no (*i.e.*, whether the foam structure of BÜTSCHLI is actually present in the walls of the large alveoli, a view which we do not put forward), there is no question at all as to the existence of such a structure in the grosser sense, and it behaves just as the finest emulsions could do. The periphery, just beneath the egg wall, exhibits a beautifully regular alveolar layer, which passes beneath into the coarser foam characteristic of the region intermediate between the periphery and the central nucleus. The protoplasm ensheathing the nucleus is more dense than the rest, but in it, too, a foam structure can be detected, at least in the early stages.

GERMINATION OF THE OOSPORE.

The nucleus of the egg, or young spore, rests, after the complete fusion of the male and female pro-nuclei, for an interval of about 20–24 hours, according to our observations. We have seen the segmentation begin sooner than this, and still more often have seen it delayed for a few hours more, but the above represents the normal period so far as our material showed, and as it was gathered at various seasons of the

* BEHRENS, "Befruchtungsvorgänge bei *Fucus vesiculosus*," Ber. d. Deutsch. Bot. Gesellsch., 1886.

† BÜTSCHLI, "Investigations on microscopic foams and on Protoplasm" (Engl. Transl.), 1894.

year, and the time was not noticeably different for the different species, it may be taken as correct for material obtained under good conditions.

Nevertheless, it is quite possible for the pre-germination period to extend much longer; thus STRASBURGER* states that the oospores obtained for him in Heligoland might remain quiescent for as long as two or three days. Perhaps this may have been due to the parent plants themselves having been exposed to unfavourable conditions, for whilst we have ourselves noticed that this factor may seriously affect the time taken over germination, we have not been able to ascertain that any very definite relation exists between the time and such external conditions as temperature (within moderate limits) and light. The condition of the sexual cells themselves at the time of fertilisation undoubtedly constitutes the prime factor in determining the rate of germination.

As the period of segmentation arrives, the nucleus itself considerably increases in size, and the shell of dense protoplasm sheathing it becomes more noticeable; it is within the latter the first indication of centrospheres is to be seen. The appearance of these bodies is somewhat irregular (figs. 24–28). Sometimes two become visible at opposite ends of the nucleus, and their feeble degree of differentiation suggests that they have arisen independently at these positions. Not unfrequently we have seen one large centrosphere at one end of the nucleus, whilst as yet none exists at the other. These cases are very striking, for the nucleus may assume a peg-top shape, the centrosphere being found at the attenuated end. In other cases, we find two centrosomes, but these are separated by an interval of less than 180°. The hypothesis that they have in these cases arisen by the fission and subsequent divarication of the halves of a single parent, is very tempting; but we do not believe that they have really been formed in this manner, since no sort of definite relation could be made out between the degree of their development and their distance from one another.

But another fact can be urged against the attempt to derive the two centrospheres from a common ancestor, namely, that in the earlier stages they are so often very unequal in size. It is sometimes possible to trace at one end of the nucleus a large and well-developed system of radiations, whilst at the other end there may as yet scarcely exist any at all, and the impression produced on the mind of the observer is that they are just arising at the latter spot. A disparity in size has been also observed in the centrosomes and radiations in some animal tissues by REINKE and others.†

We have so far spoken of the centro*sphere*, meaning by the term a series of radiations converging towards a common centre, but within this central mass minute granules may often be detected. STRASBURGER regards them as representing centrosomes, and we quite admit the tenability of this view. At the same time we do not

* STRASBURGER, "Kerntheilung u. Befruchtung bei *Fucus*," Pringsh. Jahrb. f. Wiss. Bot., vol. 30.

† REINKE, "Zellstudien," Arch. f. Mikr. Anat., vols. 63 and 64.

find sufficient uniformity, either in their number or in their general character, to feel confident in attaching much morphological importance to them. Certainly they cannot be traced in the resting cell, though their existence at the active periods of nuclear division is of interest, when it is remembered that much the same conditions prevail in some of the other plants. Thus, when the spore mother cell of the liverwort *Fossombronia** is about to divide, a simultaneous appearance of four centrospheres occurs, and, in the middle of the radiation-systems, centrosomes can be readily seen. And yet there is no evidence that these structures have been formed otherwise than at the spots at which they are first seen—certainly there is nothing to lead one to suppose that the four centrospheres have arisen by the bipartition of one original body. And furthermore, in the germinating spore of *Pellia epiphylla* the centrospheres, together with their inclusions, fade away during the protracted period of relative inactivity so characteristic of the equatorial plate stage in the karyokinensis of this plant.

But that a great range of variety in the mode of behaviour of these bodies prevails in the lower plants is shown by the fact that, according to SWINGLE,† the centrosomes themselves persist in the intervals of rest between two successive nuclear divisions in *Sphacelaria*, and thus we meet with a series of gradations extending from a relatively permanent centrosome to one which is periodically renewed, or formed afresh, at each period of nuclear division.

STRASBURGER states that in some of his own preparations he was able to trace an apparent connection between the position of the two centrospheres and the limits of the portion of the oospore nucleus which belonged to the sperm, and he suggests that this may be explained as the result of an importation, in an unrecognisable condition, of a centrosome by the sperm. Such an interpretation is tempting, in view of the statements put forward in connection with the first segmentation of animal eggs, but its foundation is weakened in *Fucus* by the fact that there is, as has been said, no visible centrosome brought in by the sperm, and also by the irregularity of its mode of appearance in the egg; for the mode mentioned by STRASBURGER is at any rate not the most frequent met with, nor, indeed, is it commonly practicable to identify the male and female parts of the nuclei for long after a normal fertilisation has taken place.

As the process of mitosis advances, the nucleus becomes richer in chromatin, and this becomes aggregated into a coiled thread, just as in the higher plants. Ultimately the coil breaks up into the definite number of chromosomes, and these are, as we have already stated in our previous communication, twice as numerous as in the gometes.

STRASBURGER gives the number for *F. serratus* as 30, but we are inclined to place

* FARMER, "On spore formation and nuclear division in the Hepaticæ," Annals of Botany, vol. 9 1895.

† SWINGLE, "Zur Kenntniss d. Kern- u. Zelltheilung b. d. Sphacelariaceen," Prings. Jahrb. f. Wiss. Bot., vol. 30.

it somewhat lower than this, and the average obtained by counting a considerable number appears to us to approximate more nearly to 26–28. In *Himanthalia*, also, of which we have good material fixed at the segmentation period, the number is likewise 28.

The peculiarity, present in the oogonial divisions, of an intra-nuclear spindle is also exhibited by the first oospore mitosis (fig. 51), and it recurs in the subsequent divisions which were studied by us. The evidence that the intra-nuclear part of the spindle is differentiated out of nuclear material which is unused for chromosome formation appears to us to be irresistible, and, although it is true that the nuclear wall thins out and finally disappears at the poles just beneath the centrospheres, a fibrillar arrangement can be discerned in the nuclear plasm before this event occurs. Thus we regard the achromatic spindle as partly nuclear and partly cytoplasmic, just as in the case of the oogonial spindles. Whilst these events have been taking place, the nucleoli disappear; they often fragment, and in any case become vacuolated, though what becomes of them we are unable to say. Possibly the various substances of which they are known to be composed are utilised for the nutrition of the nuclear constituents at these stages, and, indeed, the early vacuolation strongly suggests this. But in this plant we are unable to trace any definite relation between nucleoli and any one constituent of the nucleus. Nor is this very surprising, since even a study of the most favourable examples has led to no harmony of opinion as to the nucleolar functions, although many admit that these are at any rate largely nutritional in character. The vacuolation which points to the extraction of bodies which are or have become soluble seems to support this view.

After the differentiation of the intra-nuclear part of the spindle, the chromosomes become arrayed in its equatorial plane, and a considerable increase in the number of fibrils occurs. Whether these are due to the entrance of cytoplasm from without it is not possible to state: certainly we failed to find any evidence of *ingrowth* of new fibres. It seems not inherently improbable that cytoplasm may at this stage find its way into the nucleus, but if so it must become simultaneously differentiated into fibrils. We consider the great increase of the achromatic fibrils to be due to the differentiation of the mantle layer around the central core, the latter supplying the rigid structure, the former furnishing the mechanism for drawing off the halves of the split chromosomes to their respective poles. The fibres composing the central core are seen to possess very considerable thickness and rigidity (see fig. 52). Unlike that which occurs amongst the higher plants, the number of fibrils extending between the two groups of chromosomes, or the two daughter nuclei does not increase nor is a cell plate laid down in them (figs. 32, 33 and 53, 54). On the other hand, as the two daughter nuclei become formed, the fibrils rapidly decrease in number, and the condition of strain formerly apparent in them gives way and they gradually become slack and wavy. At the same time a change comes over the arrangement of the protoplasm surrounding the (now) inter-nuclear region of the protoplasm. The

chromatosphores gradually invade it, and although they show by the regularity with which they arrange themselves with their long axes parallel to the fibres that the state of strain is not yet over (figs. 32, 33), they do not long retain their orientation. The granular character of the protoplasm in this region slowly passes away as it assumes the normal cytoplasmic structure of the rest of the spore. The two daughter nuclei are very much smaller than the original one, and for a while the sides turned towards each other are marked by crenate protuberances (figs. 53, 54). Nucleoli make their appearance within them, and they gradually pass into the resting condition.

For some time, the radiations persist around the original poles, and they may often be seen to curve over the edges of the daughter nuclei in the sense of approaching those from the pole of the opposite nucleus. However, in neither *Fucus* nor in *Ascophyllum* is this character so prominent as it is in either *Pelvetia* or in *Himanthalia*. In the former plant it is immediately connected with the formation of the cell wall, and it is so ultimately in *Himanthalia* also, although it is not so obvious in this plant, as the spore nucleus divides several times before any cell walls are formed at all, the embryo passing thus through a syncytial stage. In *Fucus* the fertilised egg at the time of its first nuclear division may have remained spherical, or it may exhibit a prolongation on one side marking the region of the first rhizoid. When the rhizoid protuberance is visible before the first nuclear division, the latter is so correlated to it as to place its long axis in the line of the physiological axis of the cell, and after the division is completed, one daughter nucleus is found in the rhizoid, which next becomes cut off by a wall from the rest of the embryo which contains the other daughter nucleus.

The outer wall of the rhizoid rapidly becomes thickened, and its external mucilaginous layers, of which a perfectly correct figure has been given by THURET * for *F. serratus*, serves to firmly attach the embryo to its substratum. But, though the cell wall is ultimately formed between the two daughter nuclei, it is not formed as in the higher plants, for it is not formed in connection with the inter-nuclear spindle, which, as has been said, gradually disappears. *Pelvetia* offers a better object in which to study the process than does either *Fucus* or *Ascophyllum*. Whereas the inter-nuclear fibres die away, the asters at the original poles increase in size, and the radiations are seen to curve especially round the nuclei (see figs. 36–39), which appear, so to speak, to be in the way, and the mass of radiation is very dense as they pass, almost saucer-wise, round the edges of the nuclei, to be separated into individual fibres further on in their course. Finally they meet the similar system of radiations from the opposite nucleus, and in the plane where the two sets of fibres meet the cell wall is laid down. But the latter does not seem to be formed as the result of the conversions of equatorially placed swellings of the spindle fibres into cell wall, but as a pellicle arising in

* "Études Phycologiques," Pl. 14, figs. 20, 21.

the protoplasm in this region. The main obvious relation of the spindle fibres is rather a directive than a formative one.

Although, in *Fucus*, *Ascophyllum*, and *Pelvetia*, division of the nucleus is speedily followed by the formation of cell-walls, this is not the case in *Himanthalia*. Possibly the large size of the much vacuolated egg hinders a response to the nuclear stimuli until a sufficient number of them have been formed. But, at any rate, there are plenty of parallels for this behaviour. Some animal embryos retain for a time a syncytial condition in various degrees, and, again, the formation of endosperm in Angiosperms is most often associated with the postponed appearance of cell-walls.

Observations on the Physiology of Germination.

The course of germination varies greatly in different cultures of eggs.

(1) In normal cases the rhizoid rudiment appears, the oospore becoming pear-shaped, the nucleus then divides, and shortly afterwards the first division wall is formed at right angles to the long diameter of the sporeling.

(2) The nucleus divides first, the rhizoid rudiment appearing subsequently.

(3) The plant rapidly divides, but remains spherical—no sign of a rhizoid appearing even in cultures a month old.

Experiments prove conclusively that the last case described is not due to want of space, for in some cultures, where the eggs are closely packed, the rhizoids are produced in a normal manner. Neither is it due to illumination from all sides, for some cultures, though placed on white ground in a well-lighted greenhouse, produced long rhizoids. Neither is it due to insufficient illumination, for some of the lots kept in darkness showed longer rhizoids than any. An experiment described below shows that the elongation of the sporeling is retarded, if not prevented altogether, by the water being too dense. If, on the other hand, the specific gravity of the water is just a trifle less than that of normal sea-water, the appearance of the rhizoid may be accelerated.

The determination of the orientation of the rhizoid when present is only another aspect of the same question. Rosenvinge* has made several experiments to try and determine this. Our experiments were chiefly confined to trying to find the effect of strong illumination from one side. The culture vessel was placed in a box with a slit at one end to admit the light. In every instance, while the majority had originated in the side away from the light, quite a large number had rhizoids arising from other parts of the eggs. One of these experiments was tried with the eggs on a surface sloping downwards and away from the light, but the result was the same. As we feared there was some amount of light reflected from the sides of the box, another was prepared and carefully blackened inside and the experiment repeated, with still similar results, though it seemed as if the number of rhizoids arising on the shaded

* Rosenvinge, "L'organisation polaire et dorsiventrale des plantes," Rev. gén. de Bot., vol. 1, p. 123.

side was a little greater. One curious fact observed was that with strong lateral illumination the number of sporelings with two rhizoids opposite each other is much greater than in those grown in diffuse light. This might seem to indicate that light does affect the orientation, but it must be remembered that with light coming through a slit shadows will be cast by the spores themselves.

It must be borne in mind, also, that the directive action of light upon the elongating rhizoid is so great that the point of origin of the rhizoid cannot be detected, except when it is just starting. Still, some of the cultures show in a clear manner rhizoids starting in a direction at an angle with that of the incident light, then turning, so as to grow away from it. We are inclined to think that the character of the egg itself has much to do with deciding the point of origin of the rhizoid, as well as with its early or late production.

The average time taken by the spores to develop the rhizoid rudiment has been stated by us in our preliminary communication to be about 24 hours. In *Fucus serratus* the rhizoids begin to form in 18 hours, and in one case they were evident after 13 hours. When the eggs and antherozoids are derived from vigorous plants, and the receptacles are not too old, and when the extrusion has been normal, all the spores germinate quickly, and the early stages are gone through at approximately the same rate. When, however, the plants are wanting in vigour, being derived from old receptacles, or when the eggs have been forced out of the conceptacles while many are still immature, great irregularities are found both as to the time and the mode of germination; it is further found that the rate of growth is in this case much slower than in the former.

Some very striking examples have been observed of the directive effect of light on rhizoids. A spore had two rhizoids growing out of opposite sides. The two grew parallel for a distance amounting to about four times the diameter of the body of the spore. The vessel was then turned through 90°, and the two rhizoids branched at their ends—all the branches growing at right angles to the previous direction of the rhizoids and away from the light. By turning the culture vessel the rhizoids may be made to double upon themselves.

It sometimes happens, more especially in slowly-growing cultures, that sporelings of *Fucus* show indications of having delayed the formation of partition walls, until two or more nuclear divisions have taken place, a mode of procedure which it will be remembered occurs normally in *Himanthalia*.

Several of the Fucaceæ delay the production of rhizoids, and then form several at a common point—*Halidrys* is a good example of this. When *Fucus* or *Ascophyllum* spores delay the process, they frequently produce more than one, or the single one produced branches early.

A rapidly elongating rhizoid shows very clearly the development of the mucilaginous sheath. In such a case this sheath stops abruptly some distance behind the apex.

The experiments made upon the influence of alterations of density of the water upon the plants have yielded some interesting results. These may be summarized as follows :—

1. Gradual concentration of the water causes plasmolysis. This does not kill the plant; if it continues, a new wall is produced within the old one, and growth still goes on, for a time at least.
2. If sea-water of normal density is now used to replace concentrated sea-water a rapid swelling results, followed by a bursting of the wall and extrusion of contents. This sudden restoration of normal density seems to be far more injurious to the plants than a slow concentration, and this may perhaps account for the fact that *Pelvetia* refuses to grow in rock pools. We find that the germlings grow for a long time in sea-water which is allowed to partly evaporate, and if the diminution of density is brought about slowly, the plants continue to grow.

Summary.

As regards the zones inhabited by the different species of *Fucus*, these remain on the whole fairly constant under normal conditions, but they are not rigidly fixed; the environment, however, may produce modifications and deviations from the more usual arrangements.

Particulars are given respecting the seasons during which the different species form their sexual cells, and some details of biological interest affecting the production and extrusion of the latter are also appended.

In the development of oogonia the first papilla possesses the same number of chromosomes as the rest of the somatic cells. After the stalk is cut off, and during the first division of the nucleus of the oogonium itself, the number of the chromosomes has already undergone a reduction to one-half. This reduction is effected in a manner quite corresponding to that which happens in higher plants. The reduced number of chromosomes is retained throughout the rest of the oogonial divisions.

These nuclear divisions are associated with the presence of centrospheres in which centrosome-like structures can sometimes be seen. The number of the latter is, however, not constant. The protoplasm in the vicinity of the nucleus at this stage appears in the modified form of kinoplasm. We do not regard the kinoplasm as a persistent protoplasmic structure, but as forming the visible expression of a certain phase of protoplasmic activity. When that period of activity is over the kinoplasm ceases to have any existence as such, and is indistinguishable from the rest of the protoplasm. The kinoplasmic radii pass into the frothy cytoplasmic structure (see esp. fig. 44, Plate 23).

The spindle which lies between the poles is intra-nuclear, the nuclear wall persisting as an obvious membrane until late on in the division.

The first two mitoses succeed each other rapidly, and the resulting four nuclei congregate in the middle of the oogonium where they are enclosed in a denser mass of protoplasm than that filling the rest of the cell. After the definitive number of nuclei has been reached, either eight eggs are formed, or some of the nuclei undergo degeneration (*Ascophyllum*, *Pelvetia*), as has already been stated by OLTMANNS. The positions of the nuclei are determined by their mutual repulsion, and cell walls are formed in dense protoplasmic layers, which at an early period delimit the mass of each oosphere. When walls are formed they do not at once extend through to the oogonial periphery—only later do they join the innermost oogonial layer of the wall.

The oogonial wall is differentiated, except about the region of the basal pit, into first two, and later into three membranes, which we proposed to designate as eno-, meso- and endo-chite. The meso- and endo-chite are not distinguishable until a later period. The liberation of the eggs is accompanied by mucilaginous change in the layers of the wall.

Sometimes eggs are found containing more than one nucleus. These have given rise in the past to erroneous views as to the facts of fertilisation. Sometimes, also, the oospheres retain the inner layers of their walls, and then present an appearance of parthenogenetic germination; the evidence against any such parthenogenetic development is, however, cumulatively great.

The biological part played by mucilage in the process of extrusion of the oospheres is considerable, and reference may be made to the body of the paper for details.

Normally only one antherozoid enters each oosphere, although cases of polyspermy do occur. The case of *Halidrys* is especially interesting, as shedding some light on the nature of the process. Groups of antherozoids are attracted to the unfertilised eggs, and after fertilisation is effected, a definite repulsion is witnessed between the egg and the unsuccessful antherozoids. The new influence upon the latter may even entail lethal consequences.

Fertilisation in healthy material occurs with great rapidity, and the fusion of the congregating nuclei is quite complete. The fertilised egg often exhibits two nucleoli, though this number is not quite constant. The presence of a second nucleolus is probably due to the sperm, but it is formed as a dissociation product from the substance of the latter, and is not imported as such into the egg. Probably the nucleolus functions as a reserve material within the nucleus, though we do not find sufficient reason for connecting it directly with the formation of the achromatic spindle. When it is used up or is about to disappear at each karyokinesis, it first becomes vacuolated, pointing to a heterogeneous nature and, also, to the conclusion that different substances of it may be appropriated to different purposes.

We do not regard the centrospheres which are formed during the first segmentation as being derived from the sperm; there is no indication of any such structures being associated with this body. Nevertheless, this does not preclude the possibility of

their being differentiated, as the result of the stimulus given to the cell by the act of fertilisation. Cases have been observed (in *Halidrys*) of fertilisation of enucleated fragments of egg, but hitherto they have not been seen to divide, and in so far they differ from the results of observations made on certain Echinoderm eggs by Boveri.

When the egg segments, the nucleus is found to possess twice the number of chromosomes as are present in the oogonial mitoses, and this double number is retained throughout the plant, save in those divisions which lead directly to the production of sexual cells, and to which allusion has already been made.

Sometimes (commonly in *Fucus*) the first segmentation is followed by septation of the spore, but in other cases, *e.g.*, in *Himanthalia*, and exceptionally in *Fucus*, the formation of partitioning walls is postponed till after the appearance of a number of nuclei within the spore.

The actual process of germination exhibits a considerable degree of variation, which can be partly correlated with changes in the environment.

Explanation of Figures, Plates 19–24.

PLATE 19.

Fig. 1. *Ascophyllum nodosum.* Karyokinesis in oogonium mother cell (from an oblique section).

Figs. 2, 3, 4. *Ascophyllum.* Early stages of first mitosis in oogonium.

Fig. 5. *Ascophyllum.* Aster stage of first oogonial mitosis.

Fig. 6. *Fucus vesiculosus.* Same stage as the last figure.

Fig. 7. Shows the four nuclei (in *F. vesiculosus*) congregated in the centre of the oogonium and surrounded by their dense sheath.

Figs. 8–13 (Plates 19, 20). *F. vesiculosus.* Stages in final nuclear division in the oogonium.

PLATE 20.

Fig. 14. *Ascophyllum nodosum.* Shows three karyokineses going on in the last oogonial division. The fourth dividing nucleus is also indicated. This figure proves that although only four of the resulting nuclei finally persist, eight are formed, in the normal fashion, in the first instance.

Fig. 15. Polar view of a centrosphere in the last figure.

Fig. 16, *a*, *b*, *c*. *Fucus vesiculosus.* Showing the relation of the oogonial walls. The endochite and mesochite have not yet become separated.

Fig. 17. *a*, part of the periphery of oogonium of *F. vesiculosus* more highly magnified, ex. = exochite, mes. = mesochite, end. = endochite; *b* represents splitting occurring where three of the oogonial partition walls separate the oospheres.

Fig. 18. Part of the base of an oogonium of *F. vesiculosus*. *s.c.* = stalk cell; *b.p.* = basal pit; *o.w.* = wall separating oospheres.

Fig. 19. *Ascophyllum*. Oosphere just fertilised. *a*, antherozoid.

PLATE 21.

Figs. 20, 21. *Fucus vesiculosus*. Antherozoid within the oosphere lying against the nucleus.

Fig. 22. *F. vesiculosus*. Fusion of antherozoid with nucleus of oosphere.

Fig. 23. *F. vesiculosus*. An instance of polyspermy. *a*, *a*, antherozoids.

Figs. 24–33 (Plates 21, 22). Germination of *F. vesiculosus*. The first nuclear division, all within 30 hours after fertilisation.

PLATE 22.

Figs. 34, 35. *Ascophyllum*. End of first nuclear division in germinating spore.

PLATE 23.

Fig. 36. *Pelvetia canaliculata*. First nuclear division in the spore. *c*, the cell plate.

Figs. 37, 38. Second division in preceding plant.

Fig. 39. Third division in same.

Fig. 40. Multicellular embryo of *Ascophyllum*.

Photomicrographs.

All (except fig. 45) magnified about 1,000 diameters.

Figs. 41–44. Stages in the last nuclear division of the nucleus in the oogonium of *Fucus vesiculosus*.

PLATE 24.

Fig. 45. Divided oogonium of *F. vesiculosus*, one oosphere containing two nuclei. (× 600.)

Fig. 46. Portion of oogonium extruded amongst mature eggs, showing two nuclei in focus.

Figs. 47–49. Stages in fertilisation of *Ascophyllum nodosum*.

Figs. 50–54. *Fucus vesiculosus*. First nuclear division in the oospore.

Fig. 55. *F. vesiculosus*. Oospore with rhizoid and two nuclei.

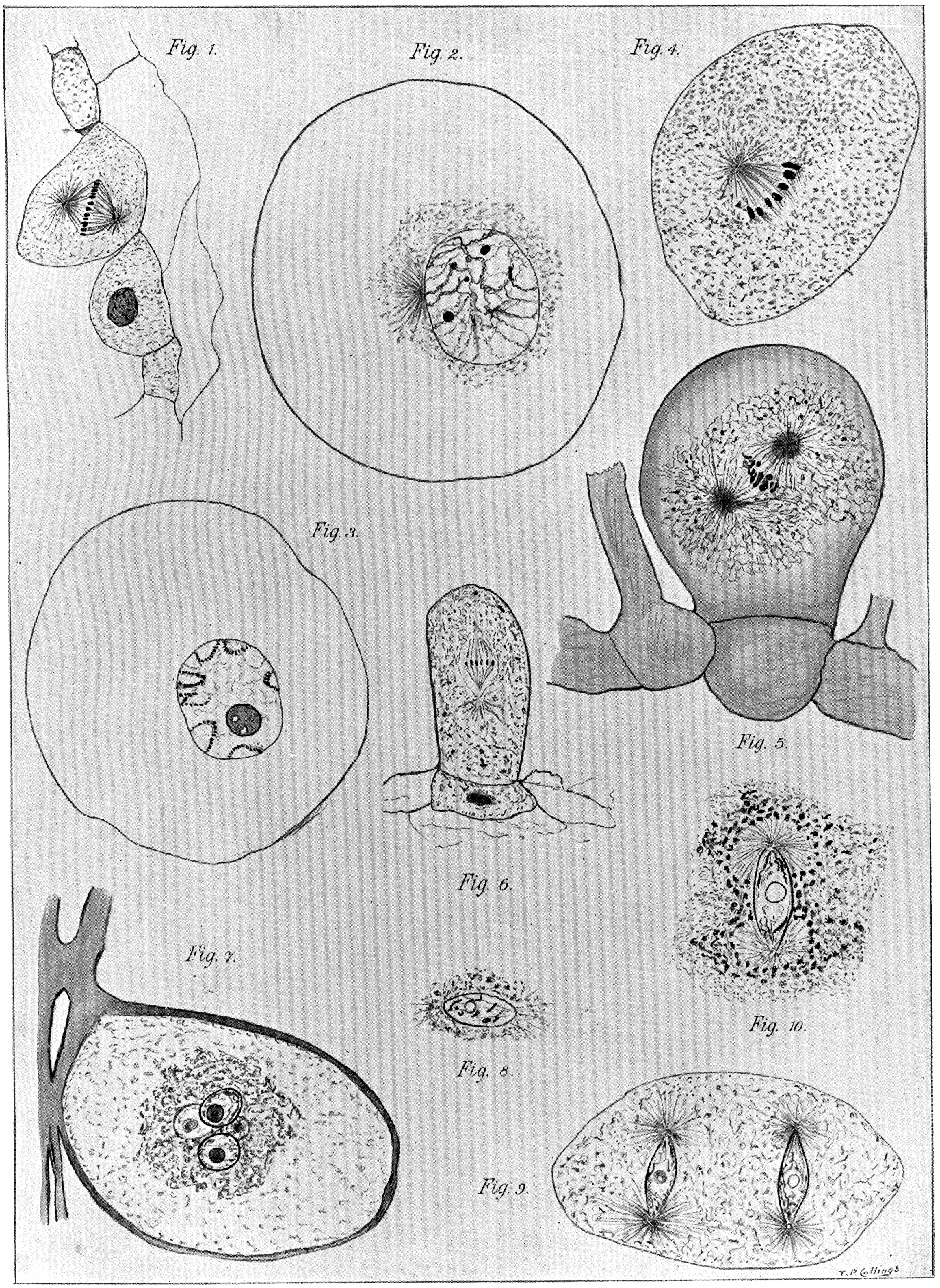
Fig. 1.
Fig. 2.
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T. P. & C. Hings

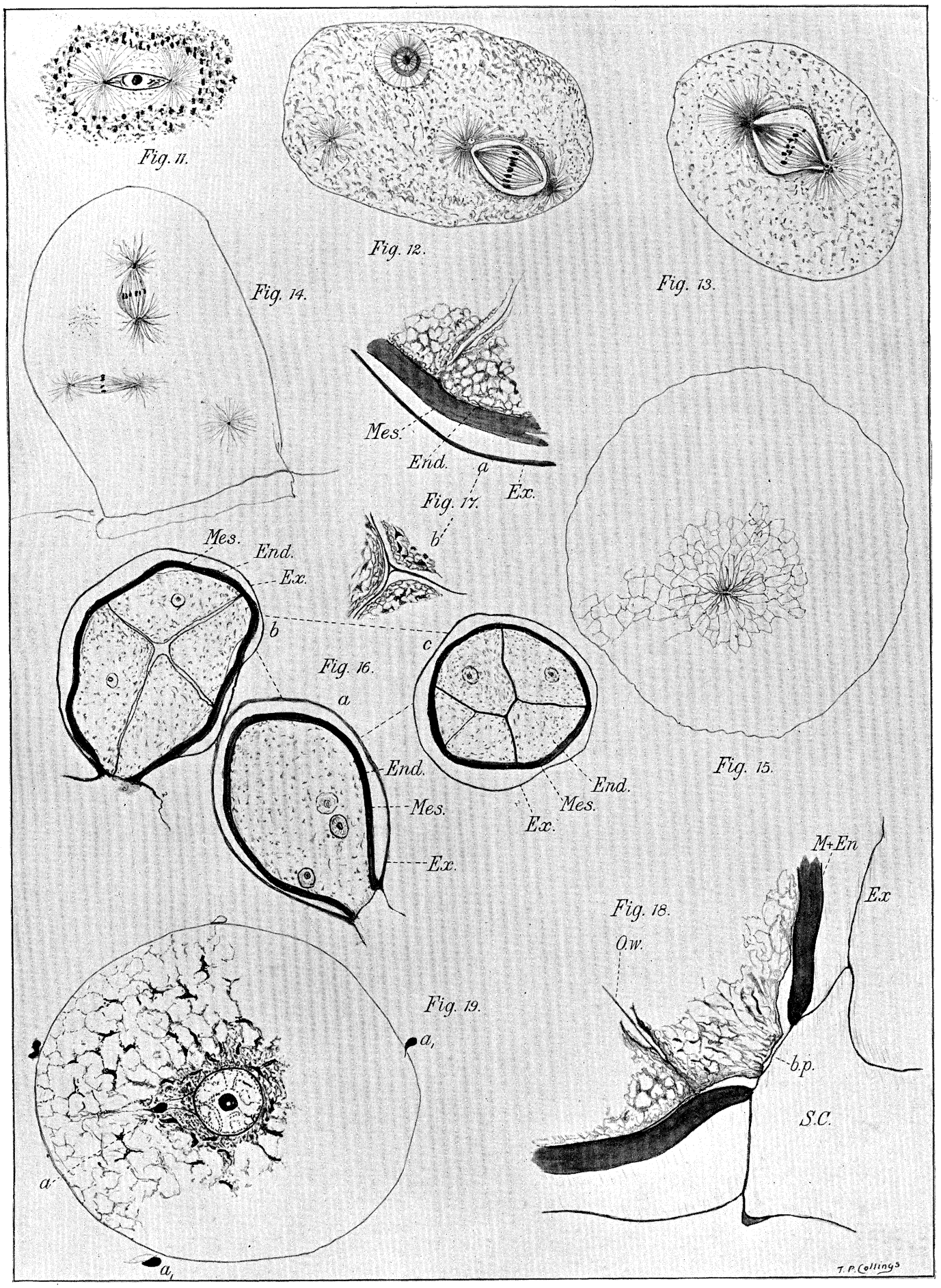
Fig. 11.
Fig. 12.
Fig. 13.
Fig. 14.
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Fig. 17.
b'
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Fig. 16.
a
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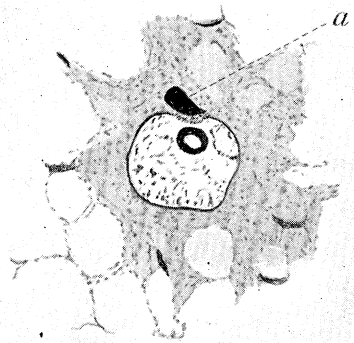
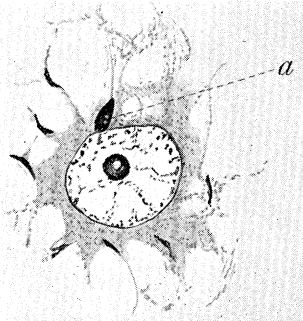


Fig. 20.



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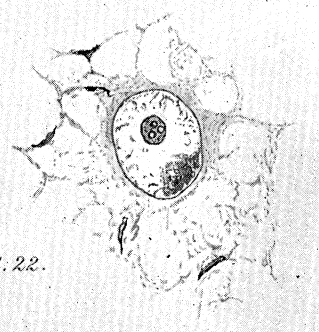

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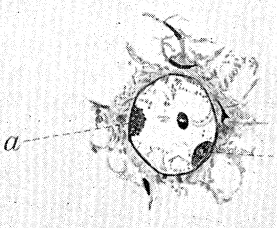

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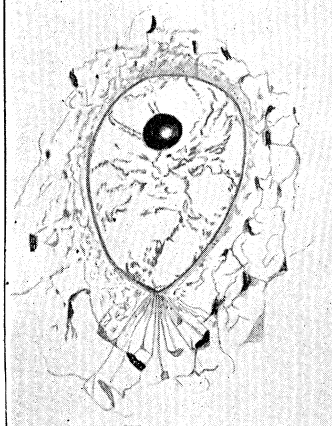

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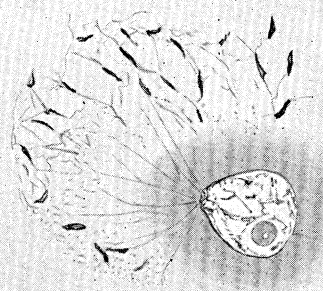

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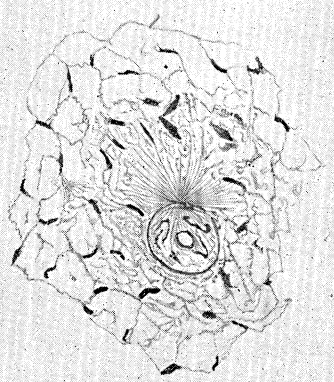

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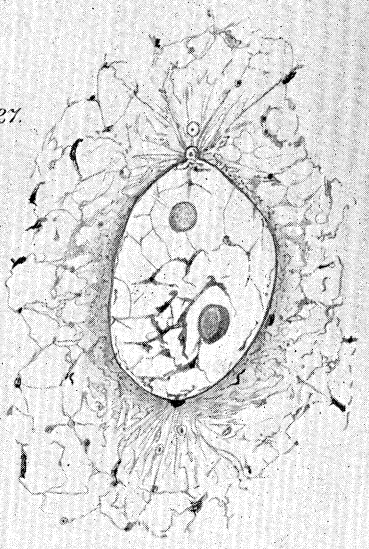

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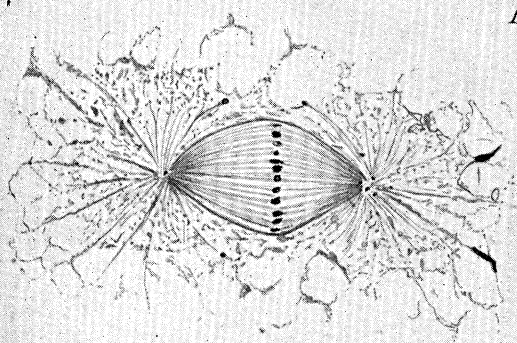

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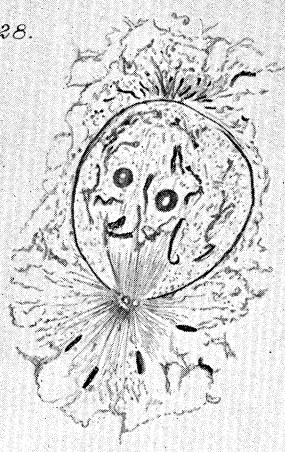

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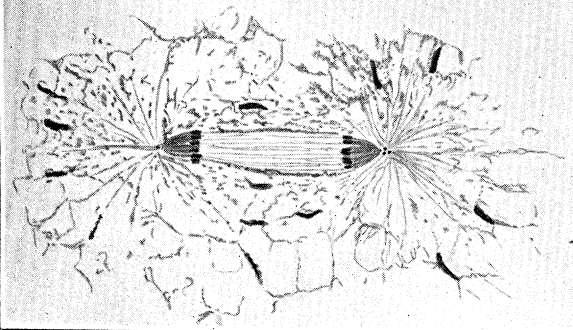

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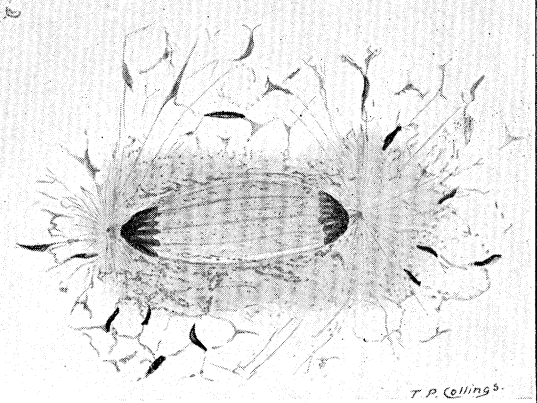


Fig. 31.

T. P. Collings.

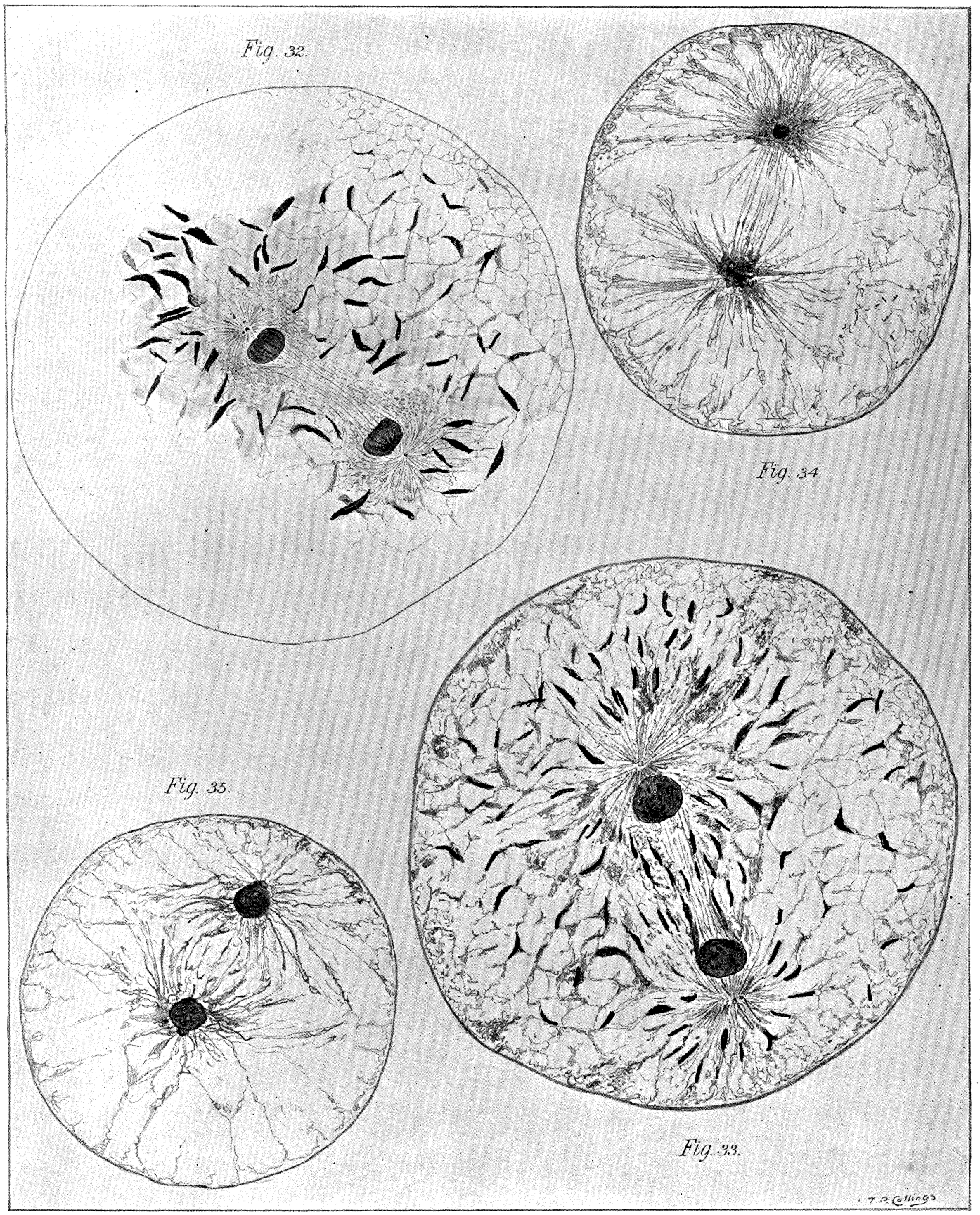
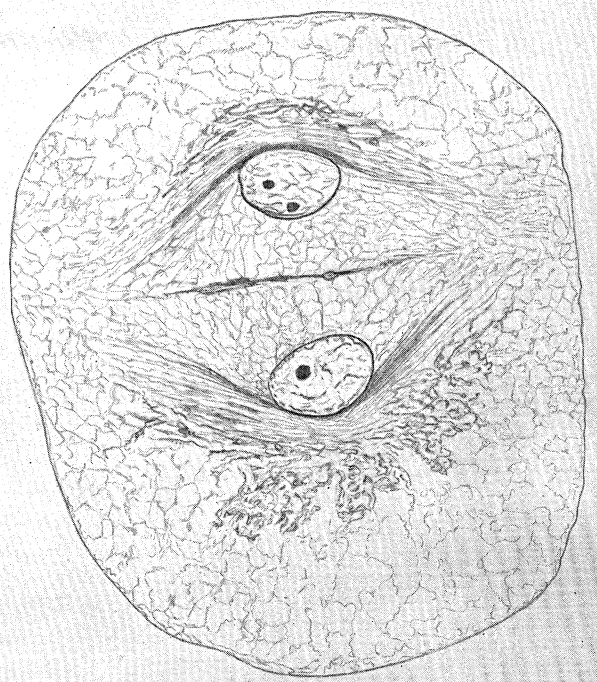


Fig. 32.
Fig. 34.
Fig. 35.
Fig. 33.



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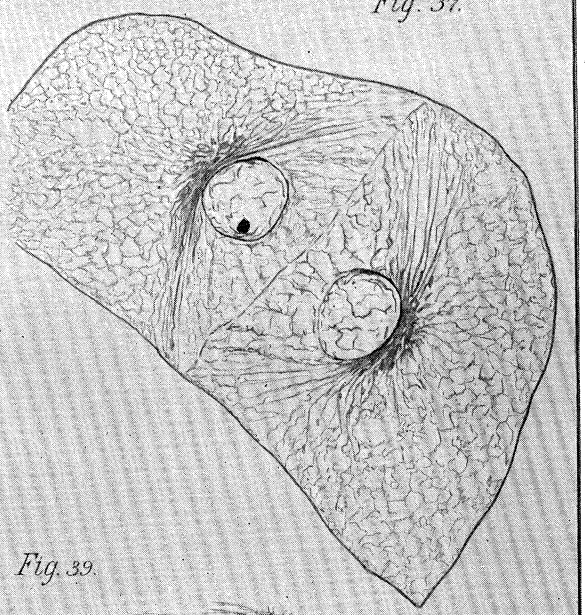

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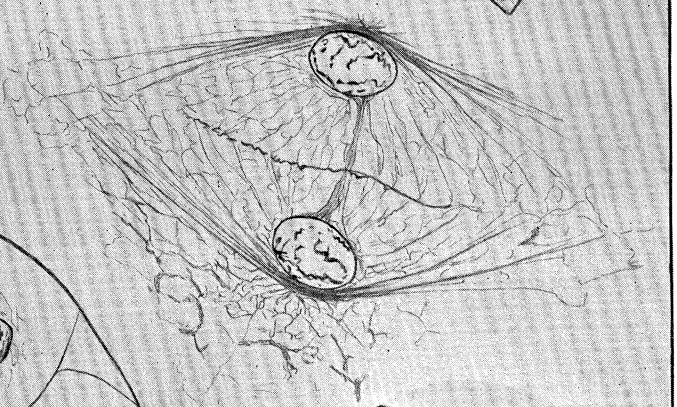

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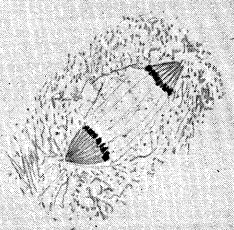

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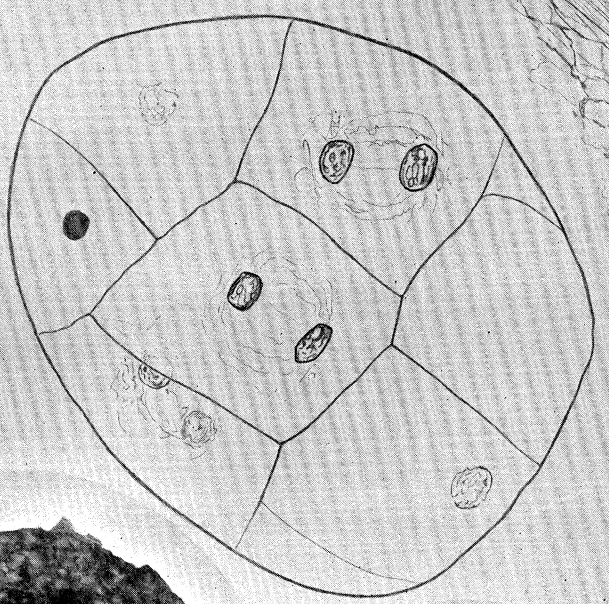
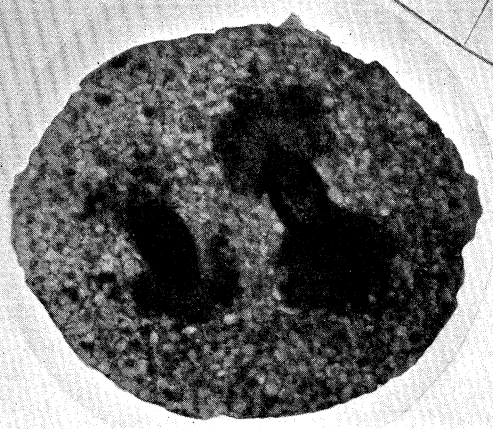


Fig. 40.

Fig. 42.

Fig. 41.



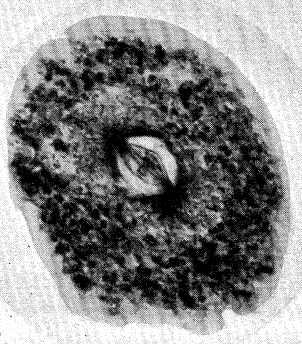


Fig. 43.

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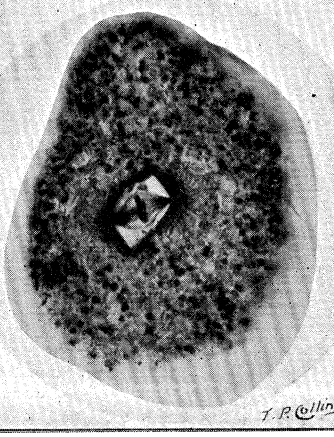

T. R. Collings

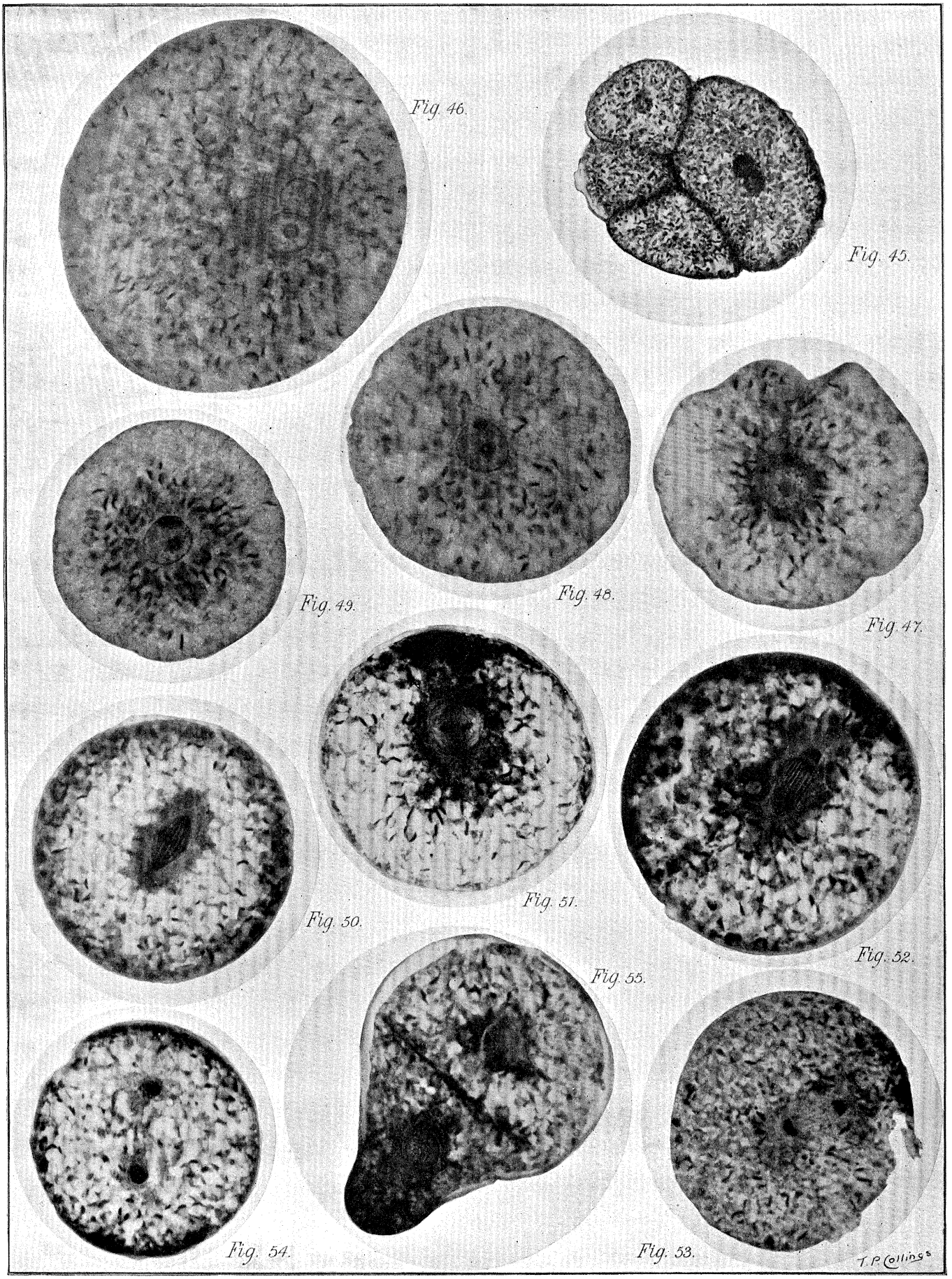
Fig. 46.
Fig. 45.
Fig. 49.
Fig. 48.
Fig. 47.
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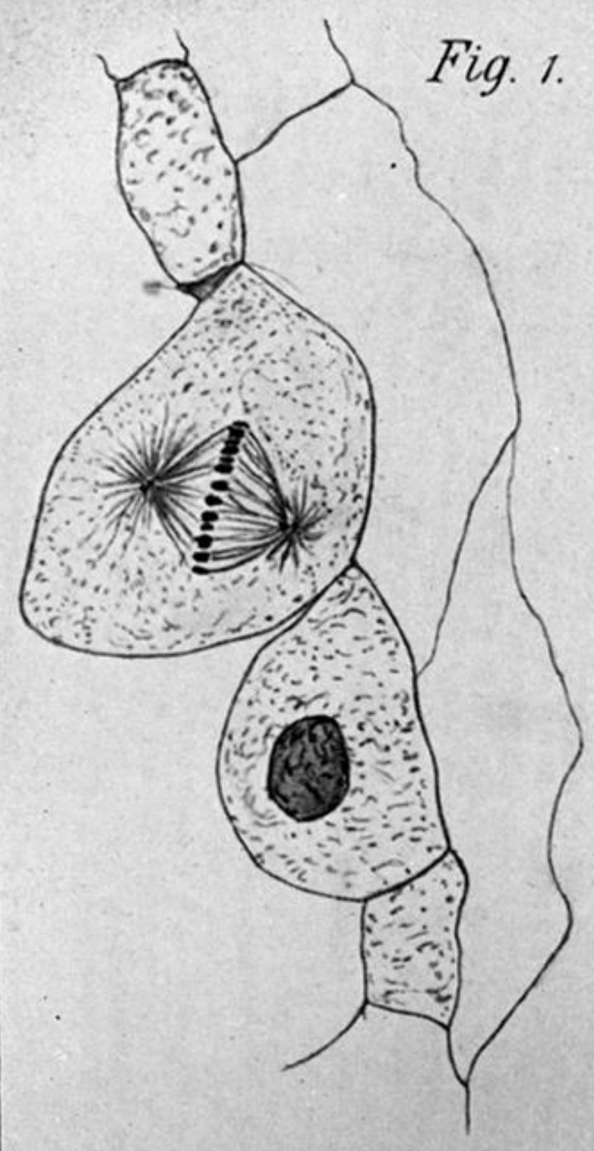
Fig. 54.
Fig. 53.
T. P. Collings



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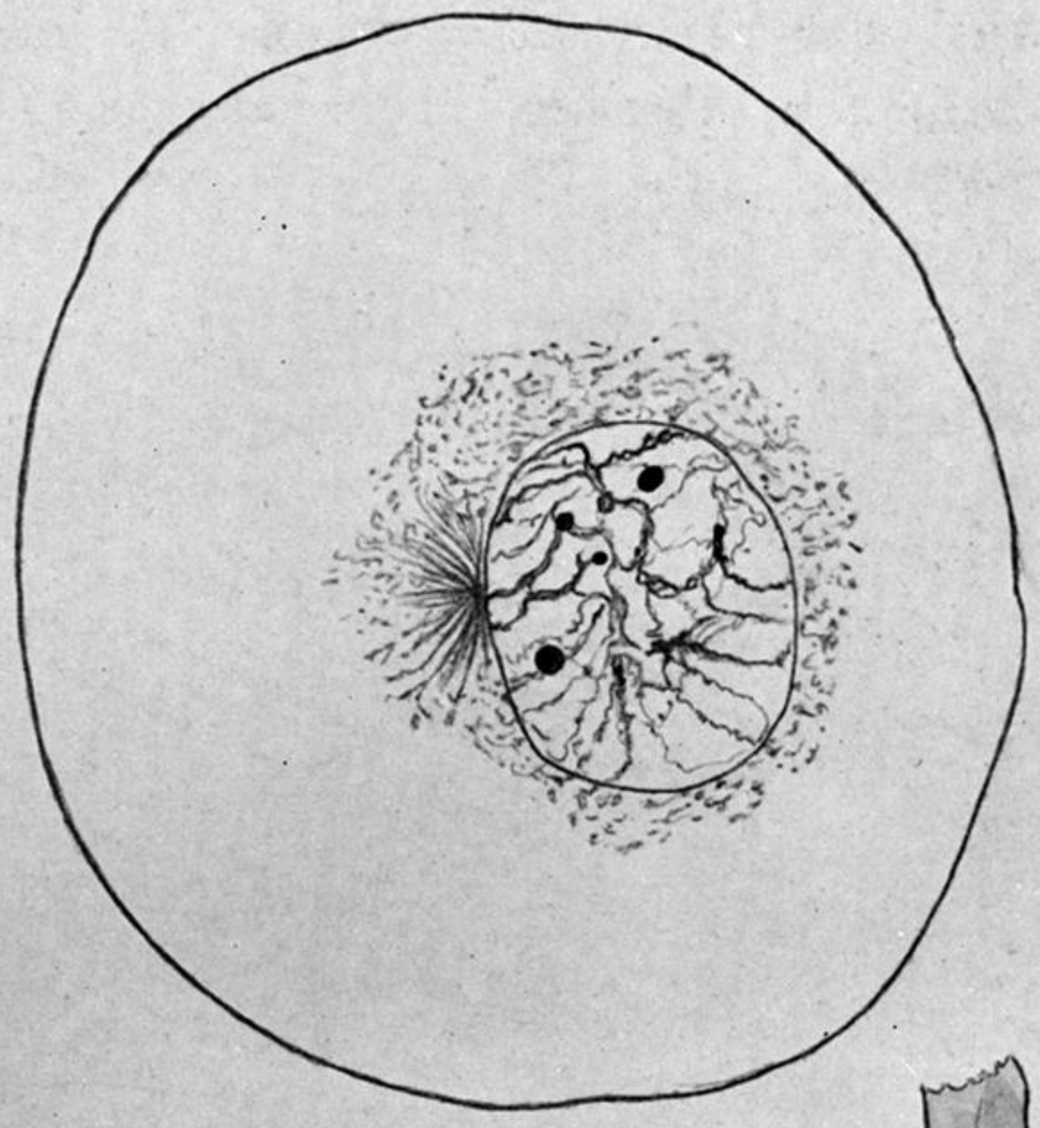

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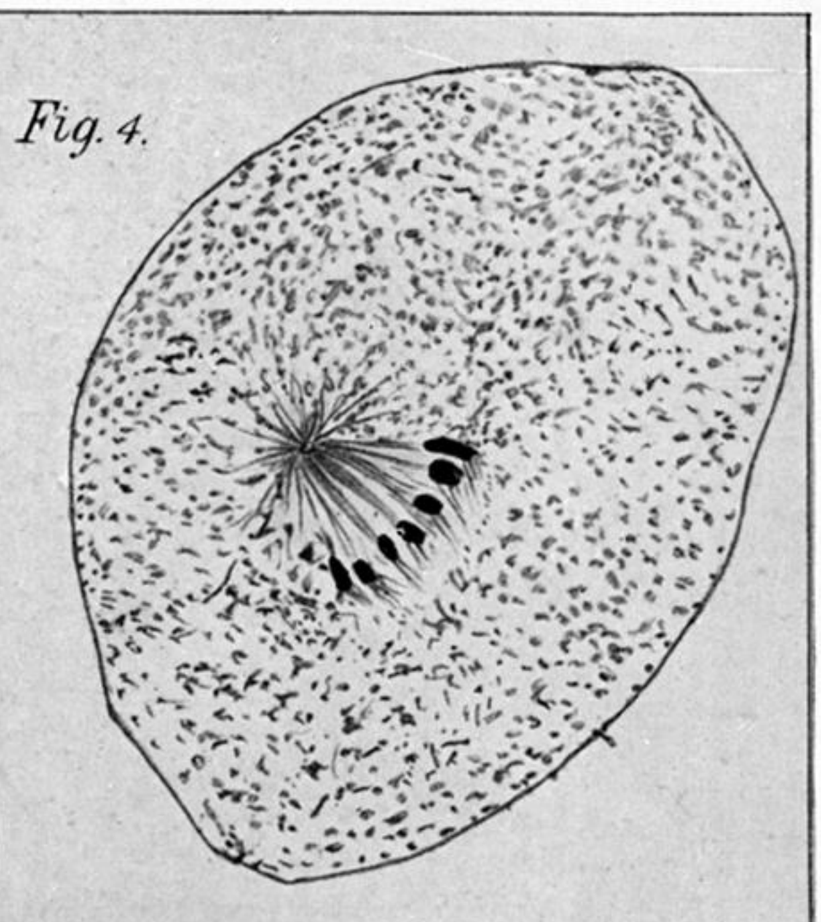
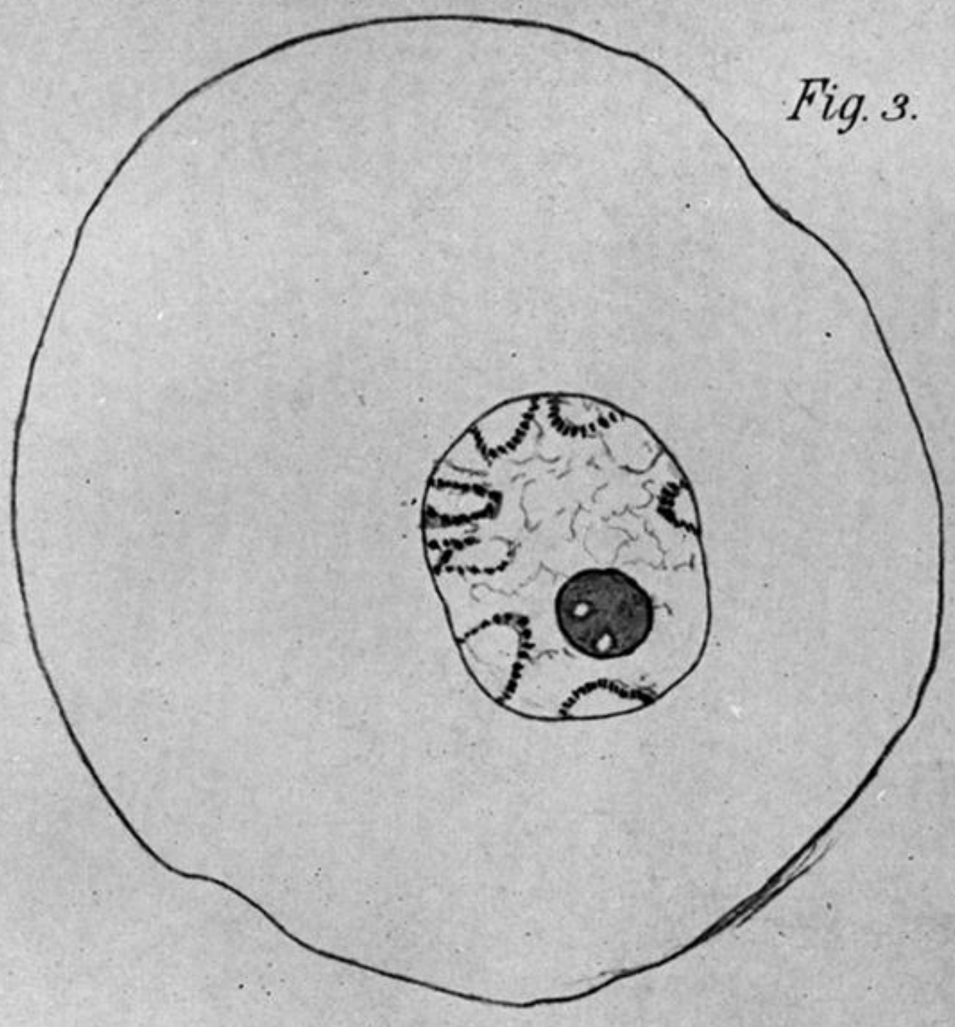


Fig. 4.

Fig. 5.



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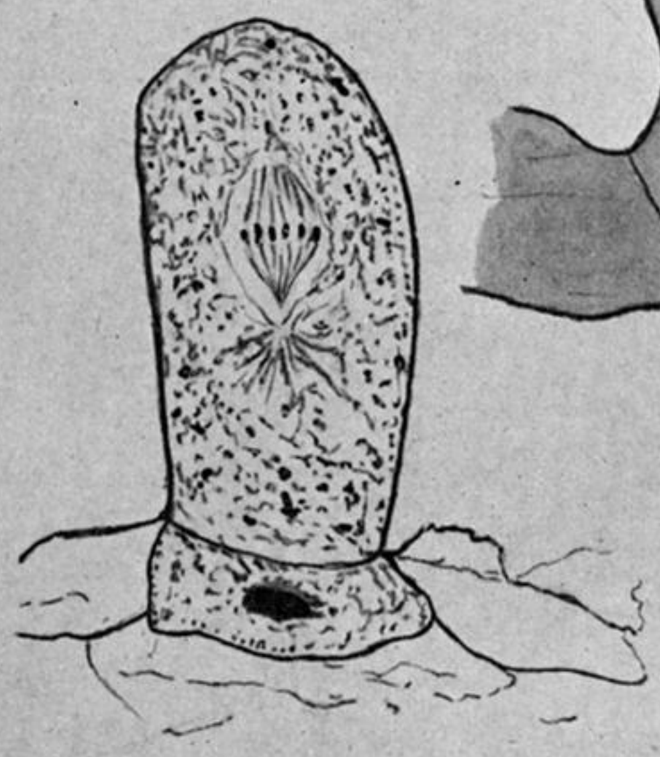
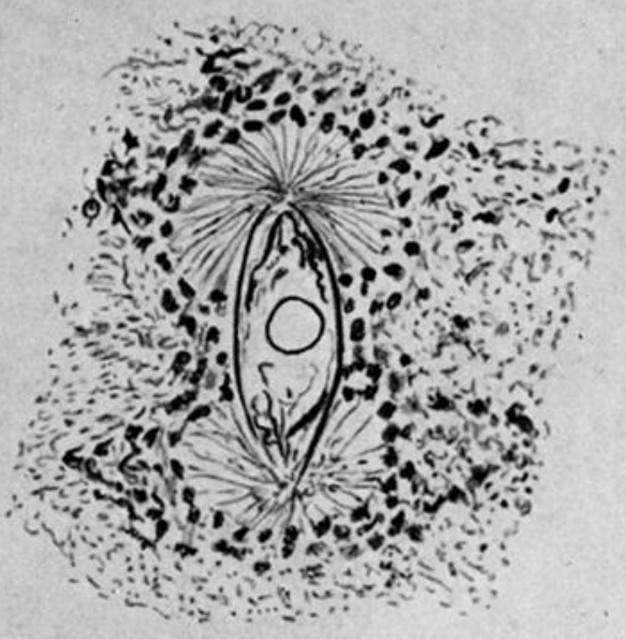


Fig. 6.



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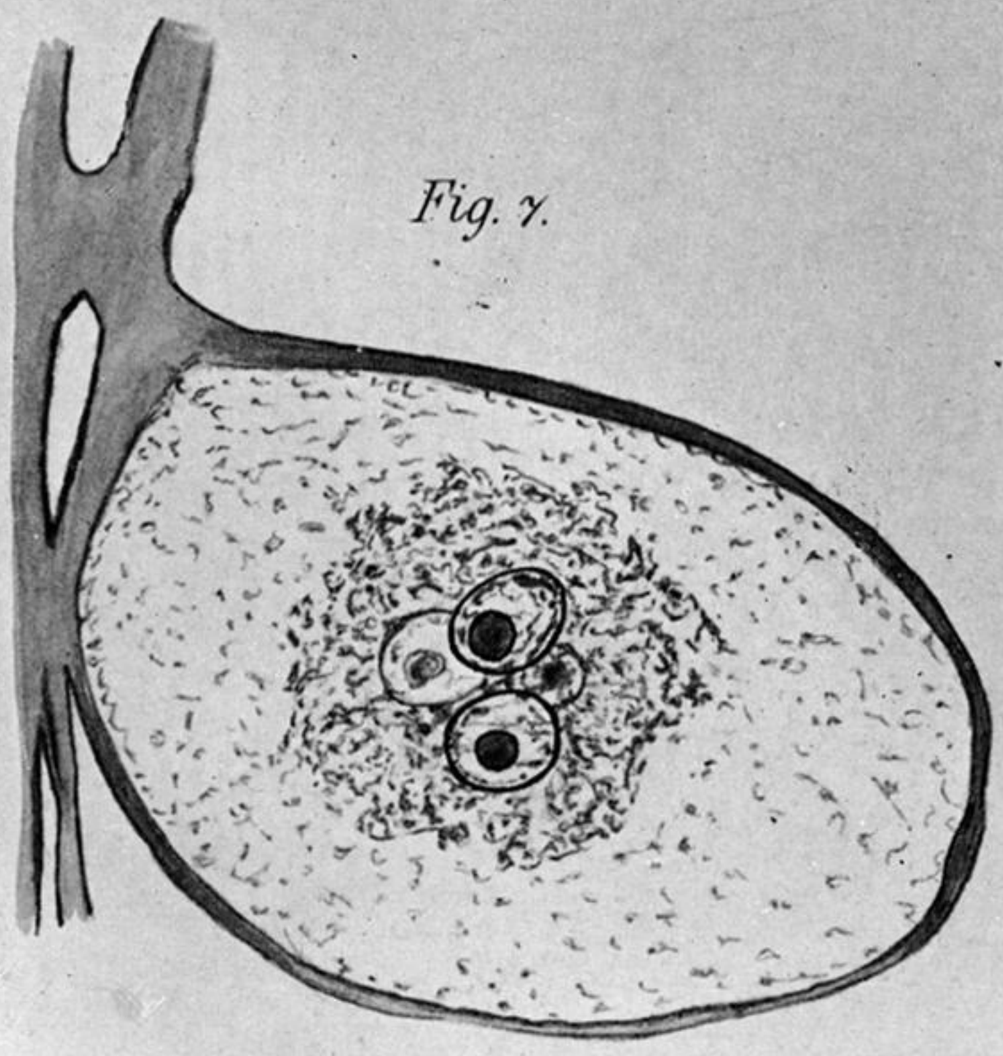

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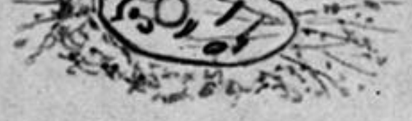

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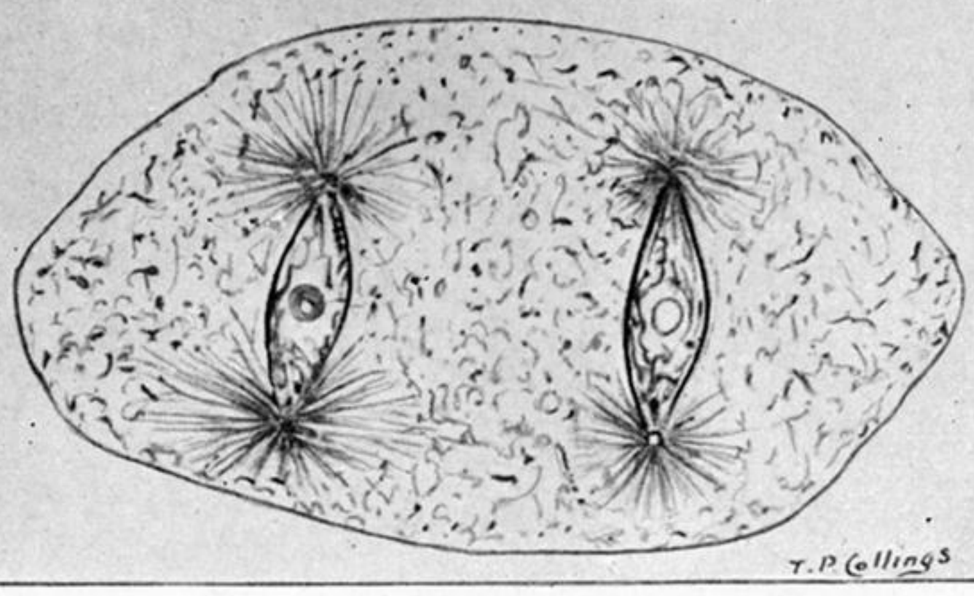


Fig. 9.

T. P. Collings

PLATE 19.

Fig. 1. *Ascophyllum nodosum.* Karyokinesis in oogonium mother cell (from an oblique section).

Figs. 2, 3, 4. *Ascophyllum.* Early stages of first mitosis in oogonium.

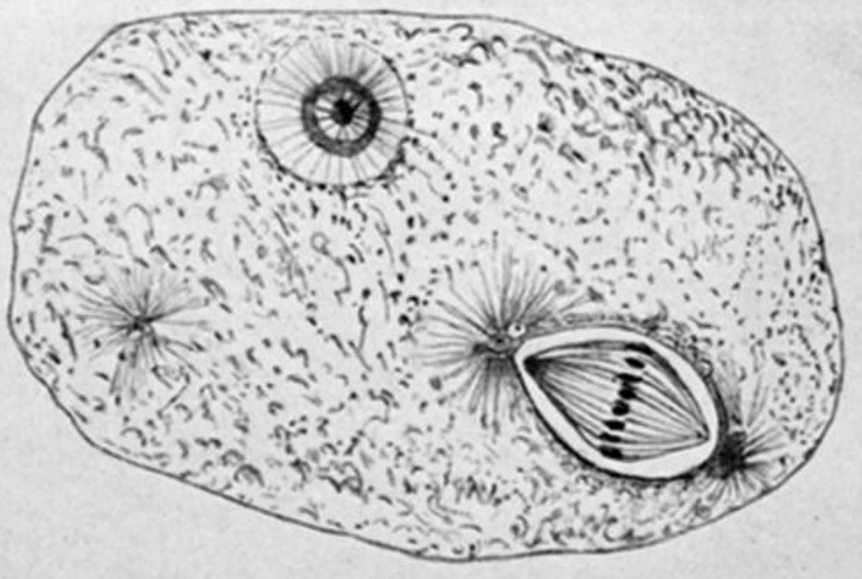
Fig. 5. *Ascophyllum.* Aster stage of first oogonial mitosis.

Fig. 6. *Fucus vesiculosus.* Same stage as the last figure.

Fig. 7. Shows the four nuclei (in *F. vesiculosus*) congregated in the centre of the oogonium and surrounded by their dense sheath.

Figs. 8–13 (Plates 19, 20). *F. vesiculosus.* Stages in final nuclear division in the oogonium.

Fig. 11.



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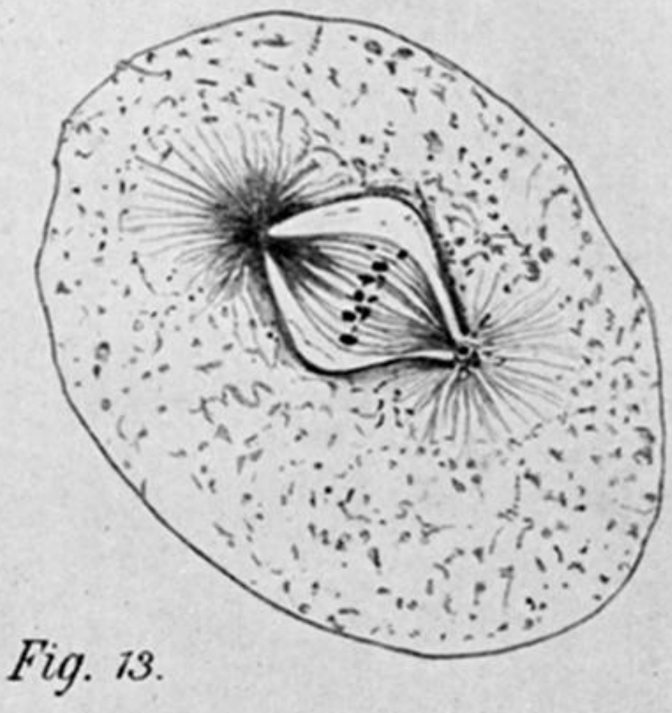

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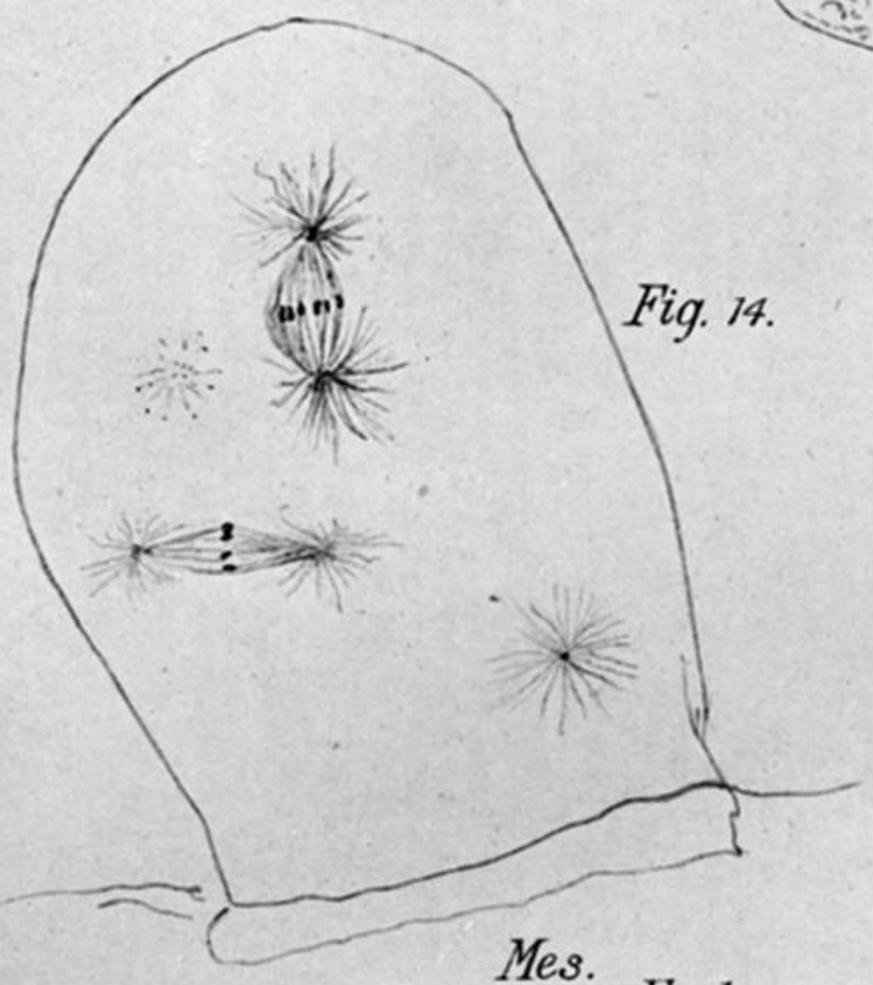

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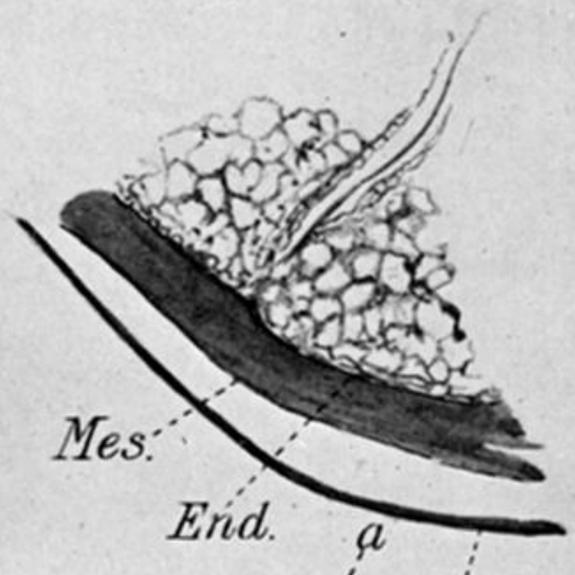

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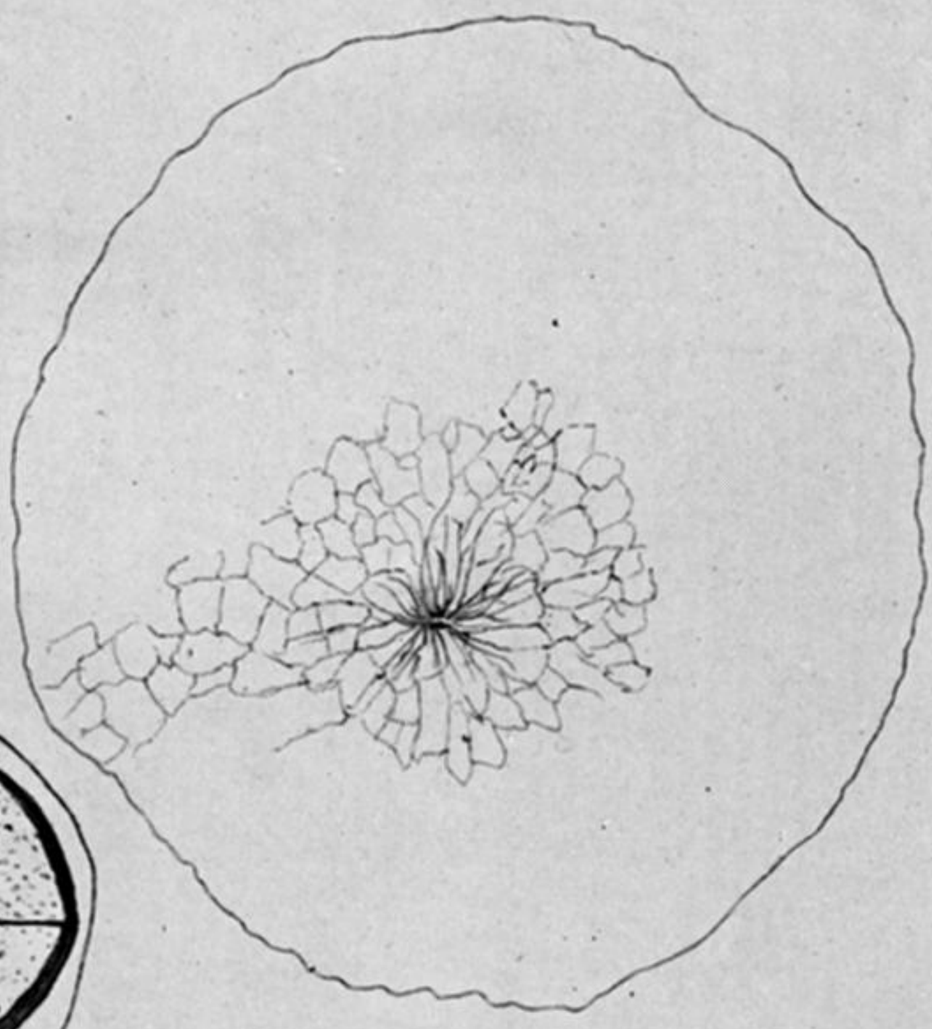

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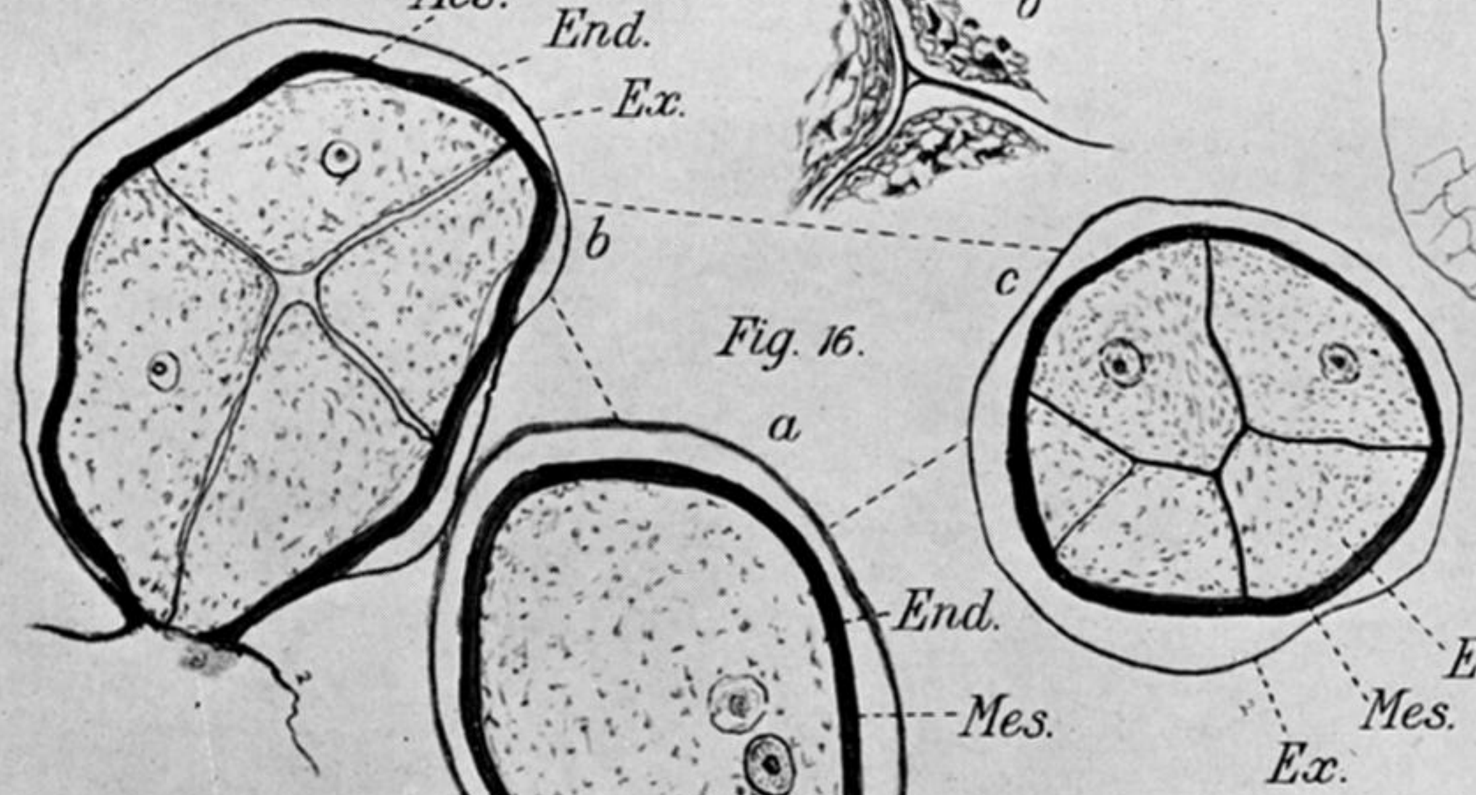

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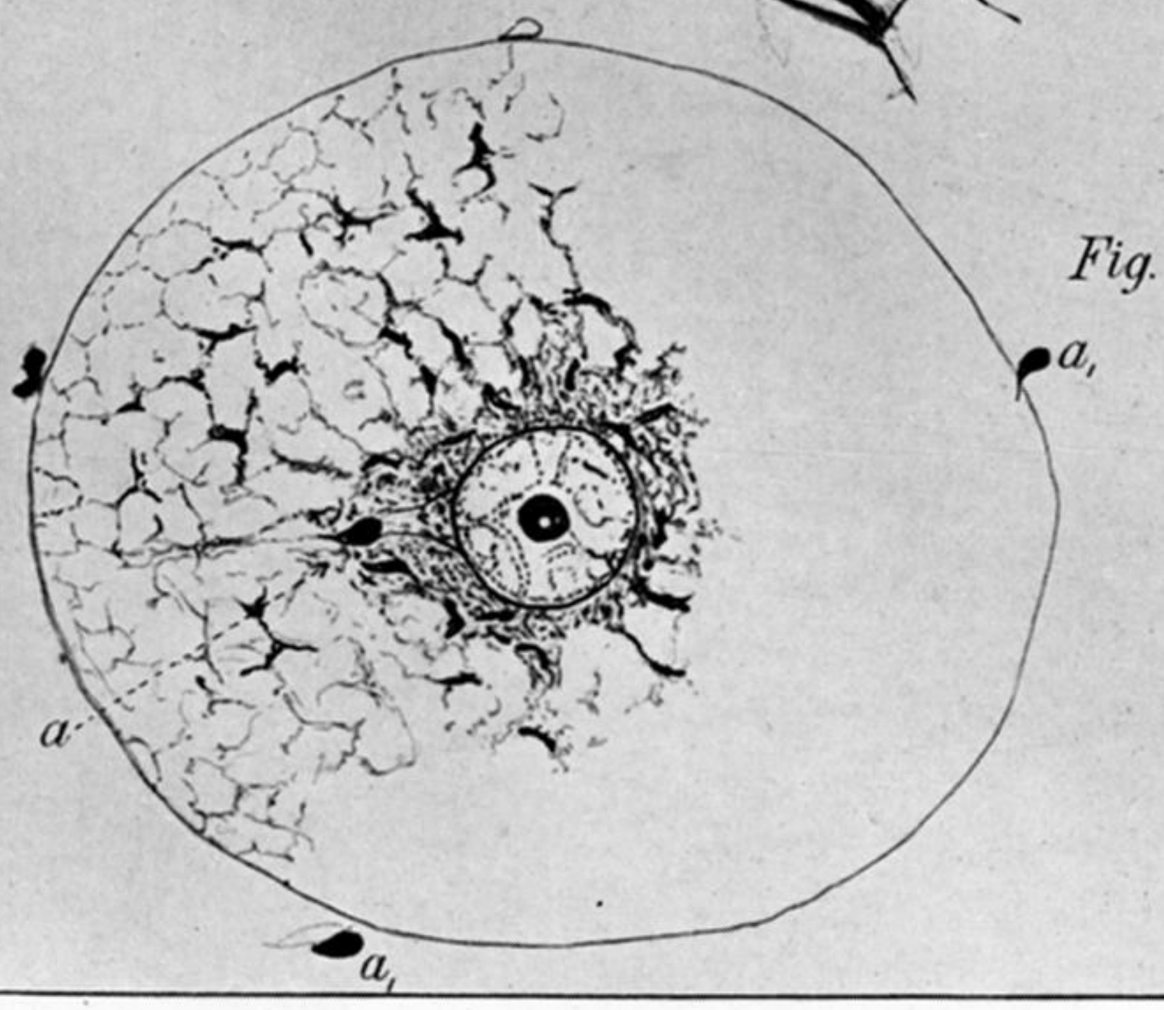


Fig. 19.

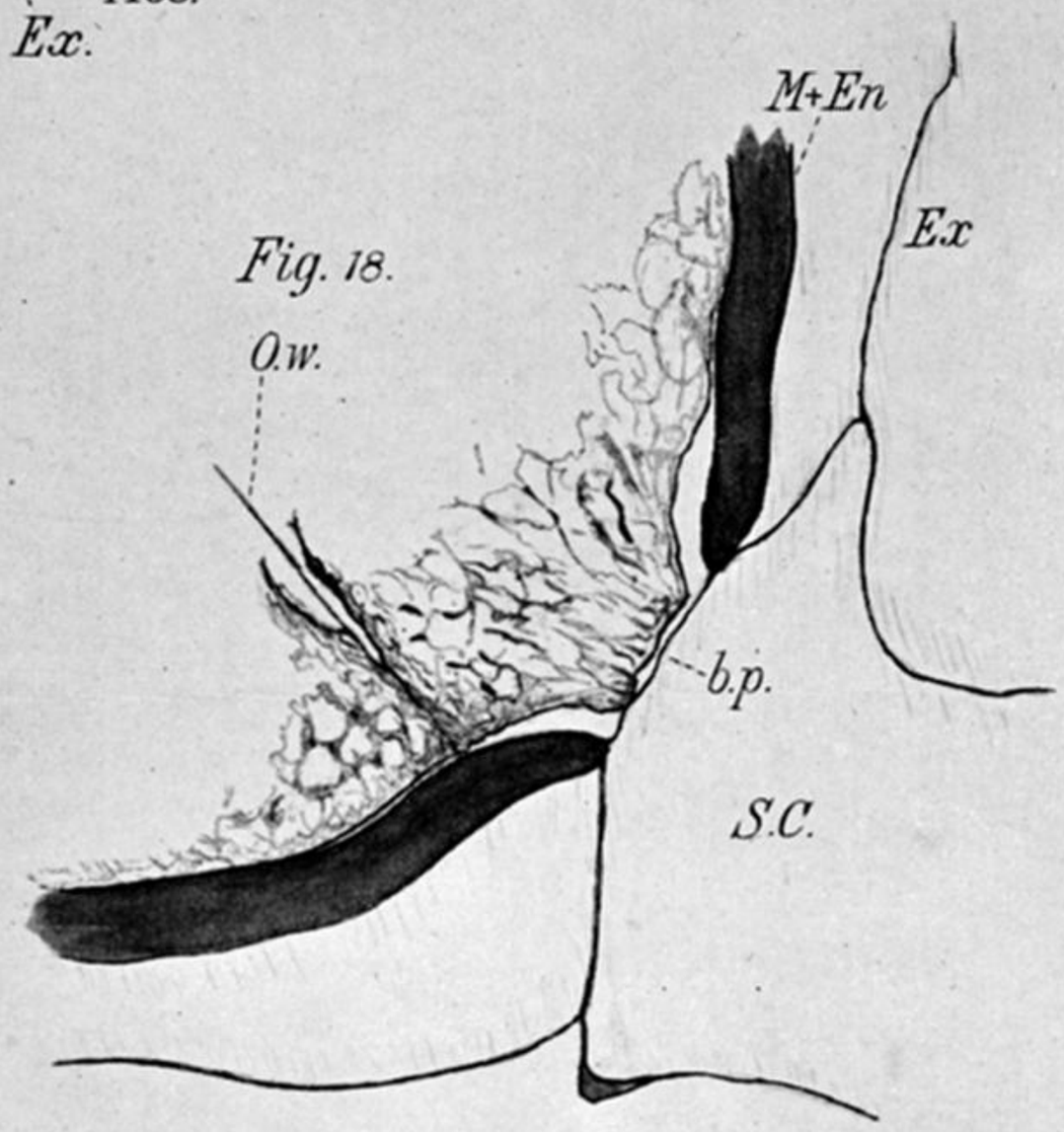


Fig. 18.

PLATE 20.

Fig. 14. *Ascophyllum nodosum.* Shows three karyokineses going on in the last oogonial division. The fourth dividing nucleus is also indicated. This figure proves that although only four of the resulting nuclei finally persist, eight are formed, in the normal fashion, in the first instance.

Fig. 15. Polar view of a centrosphere in the last figure.

Fig. 16, *a*, *b*, *c*. *Fucus vesiculosus.* Showing the relation of the oogonial walls. The endochite and mesochite have not yet become separated.

Fig. 17. *a*, part of the periphery of oogonium of *F. vesiculosus* more highly magnified, ex. = exochite, mes. = mesochite, end. = endochite; *b* represents splitting occurring where three of the oogonial partition walls separate the oospheres.

Fig. 18. Part of the base of an oogonium of *F. vesiculosus.* *s.c.* = stalk cell; *b.p.* = basal pit; *o.w.* = wall separating oospheres.

Fig. 19. *Ascophyllum.* Oosphere just fertilised. *a*, antherozoid.

Fig. 20.

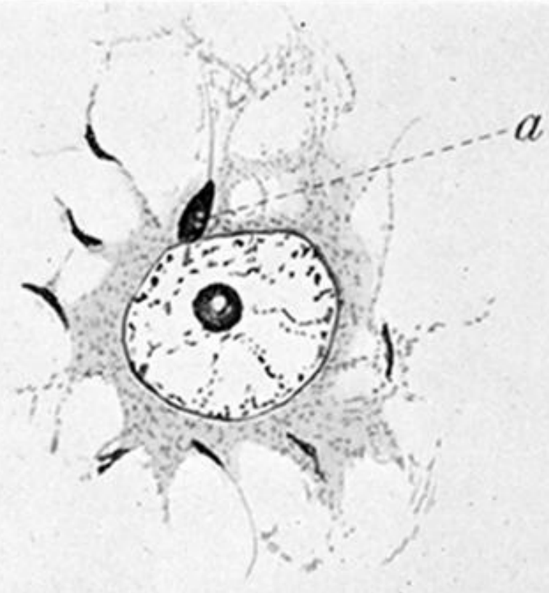
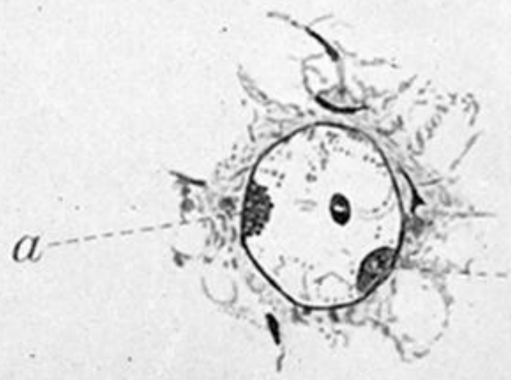


Fig. 21.

Fig. 22.



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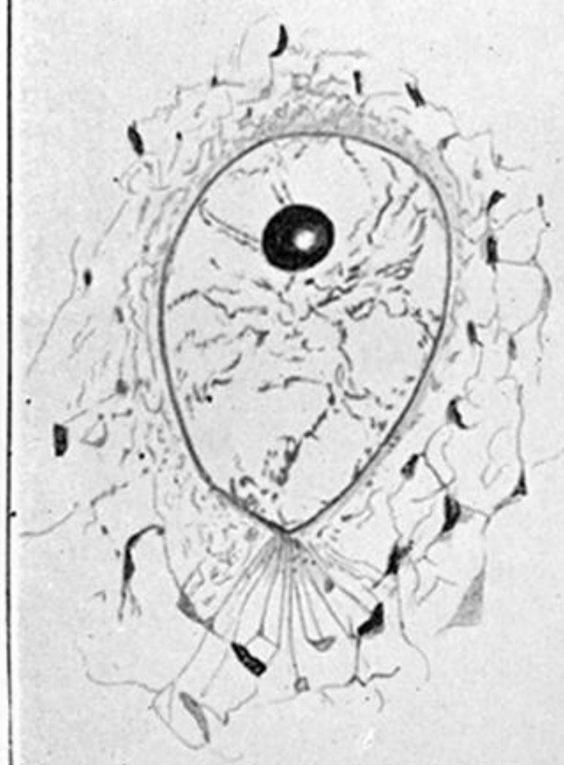


Fig. 24.

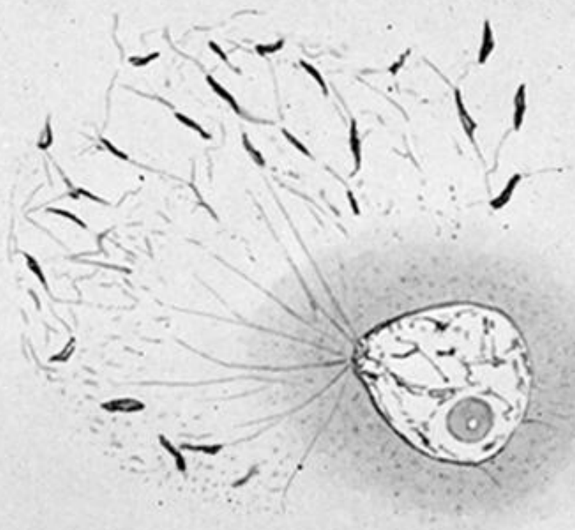


Fig. 26.

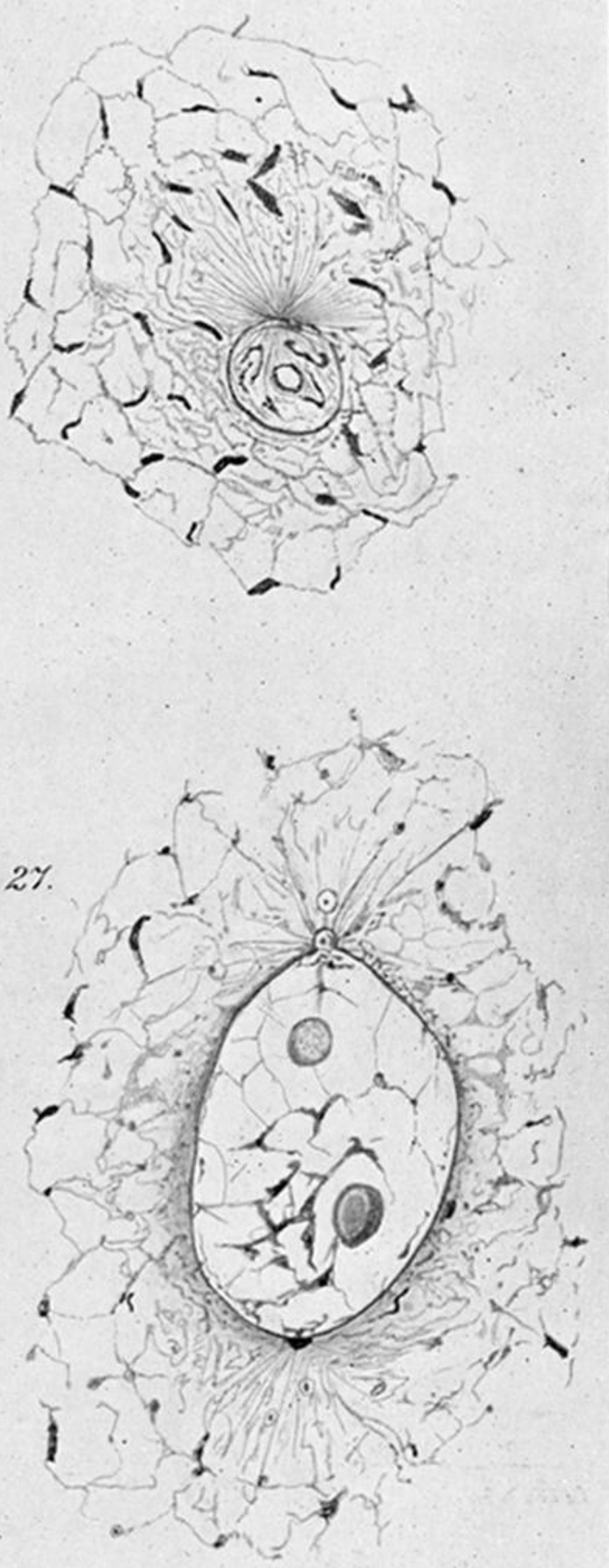


Fig. 25.

Fig. 27.

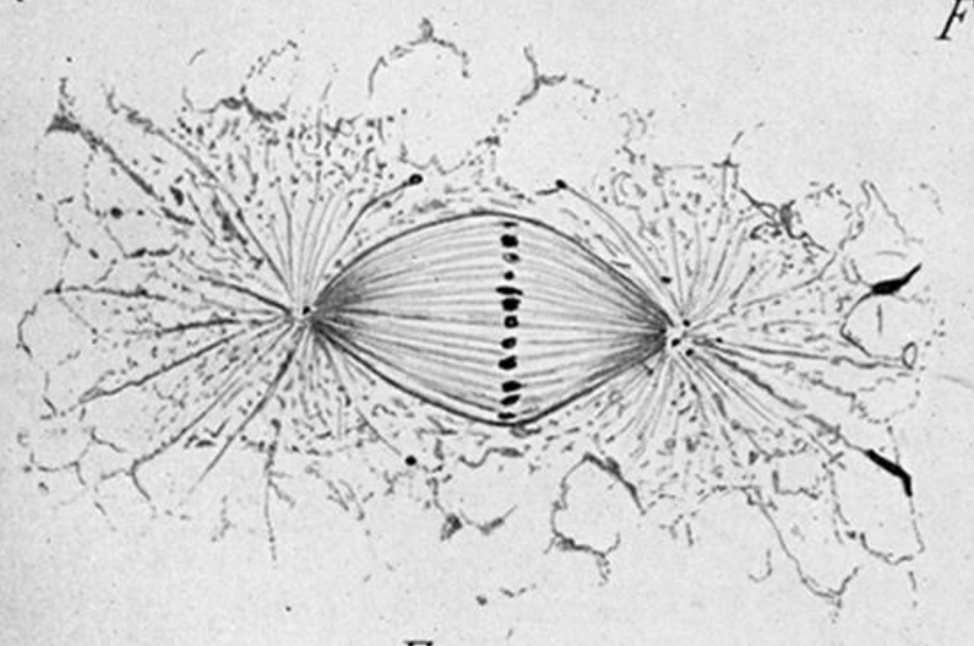
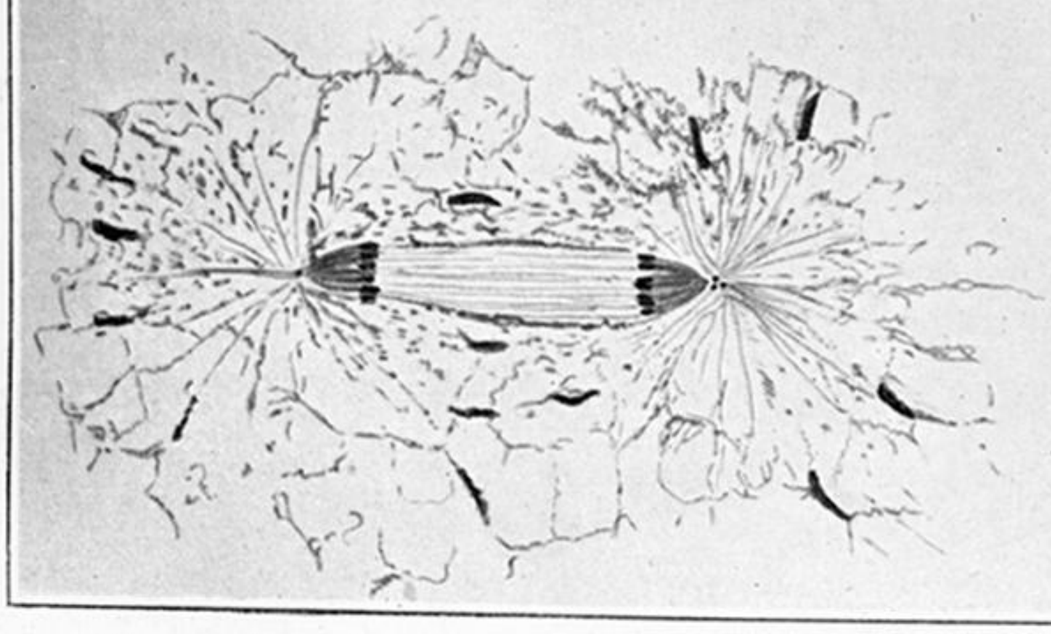


Fig. 28.

Fig. 29.



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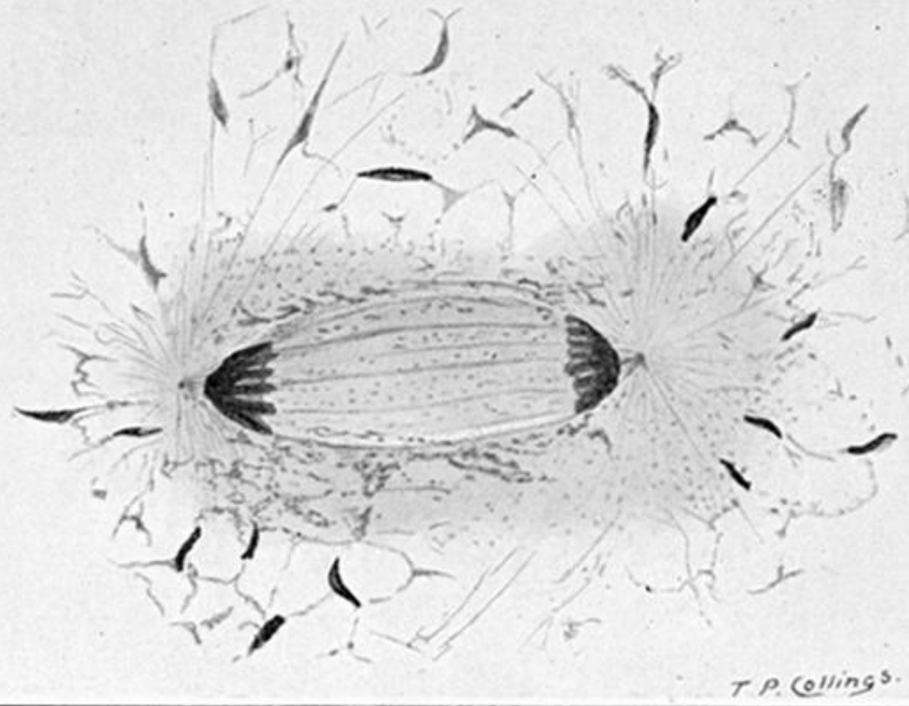


Fig. 31.

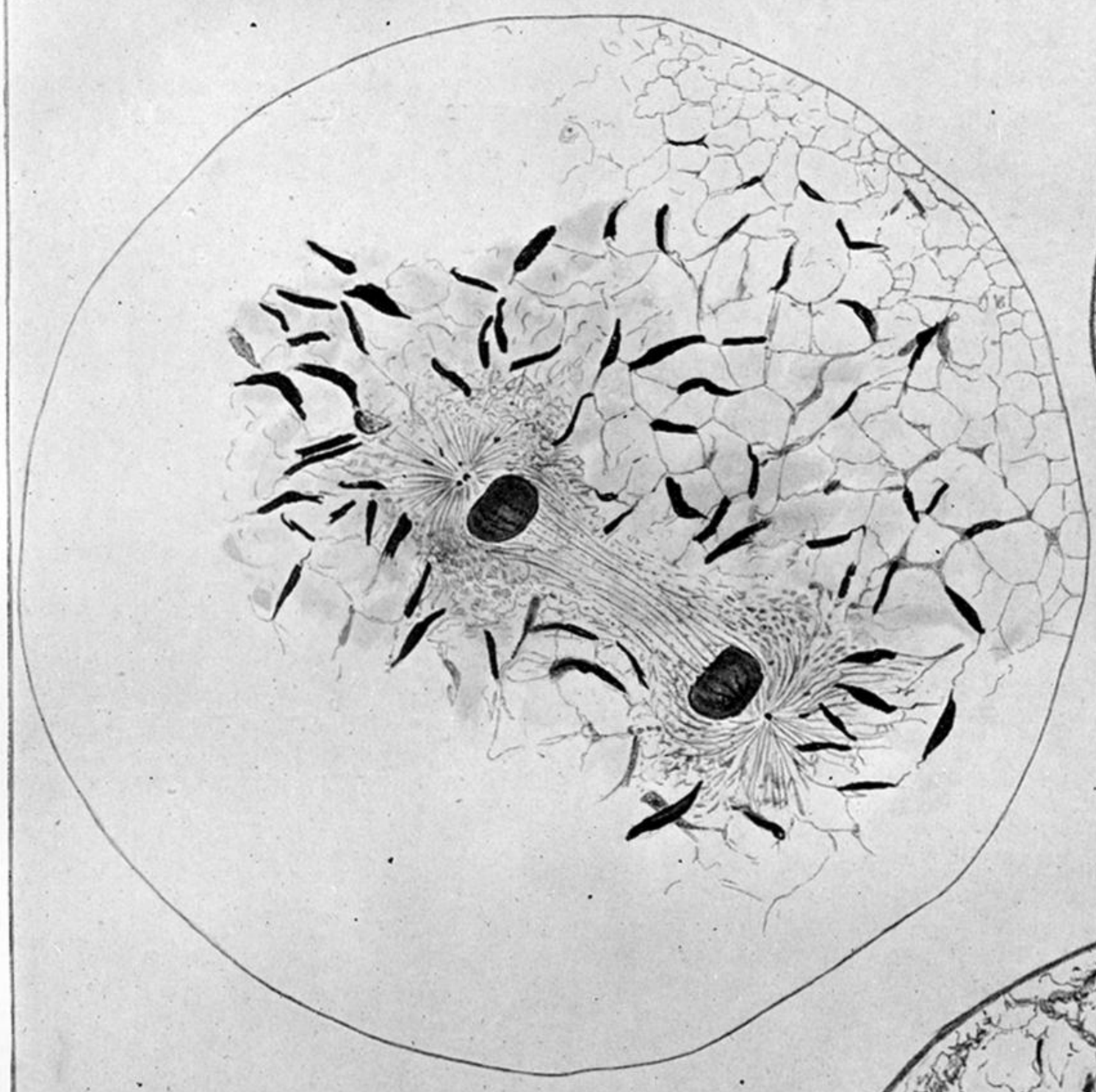
PLATE 21.

Figs. 20, 21. *Fucus vesiculosus.* Antherozoid within the oosphere lying against the nucleus.

Fig. 22. *F. vesiculosus.* Fusion of antherozoid with nucleus of oosphere.

Fig. 23. *F. vesiculosus.* An instance of polyspermy. *a*, *a*, antherozoids.

Figs. 24–33 (Plates 21, 22). Germination of *F. vesiculosus.* The first nuclear division, all within 30 hours after fertilisation.



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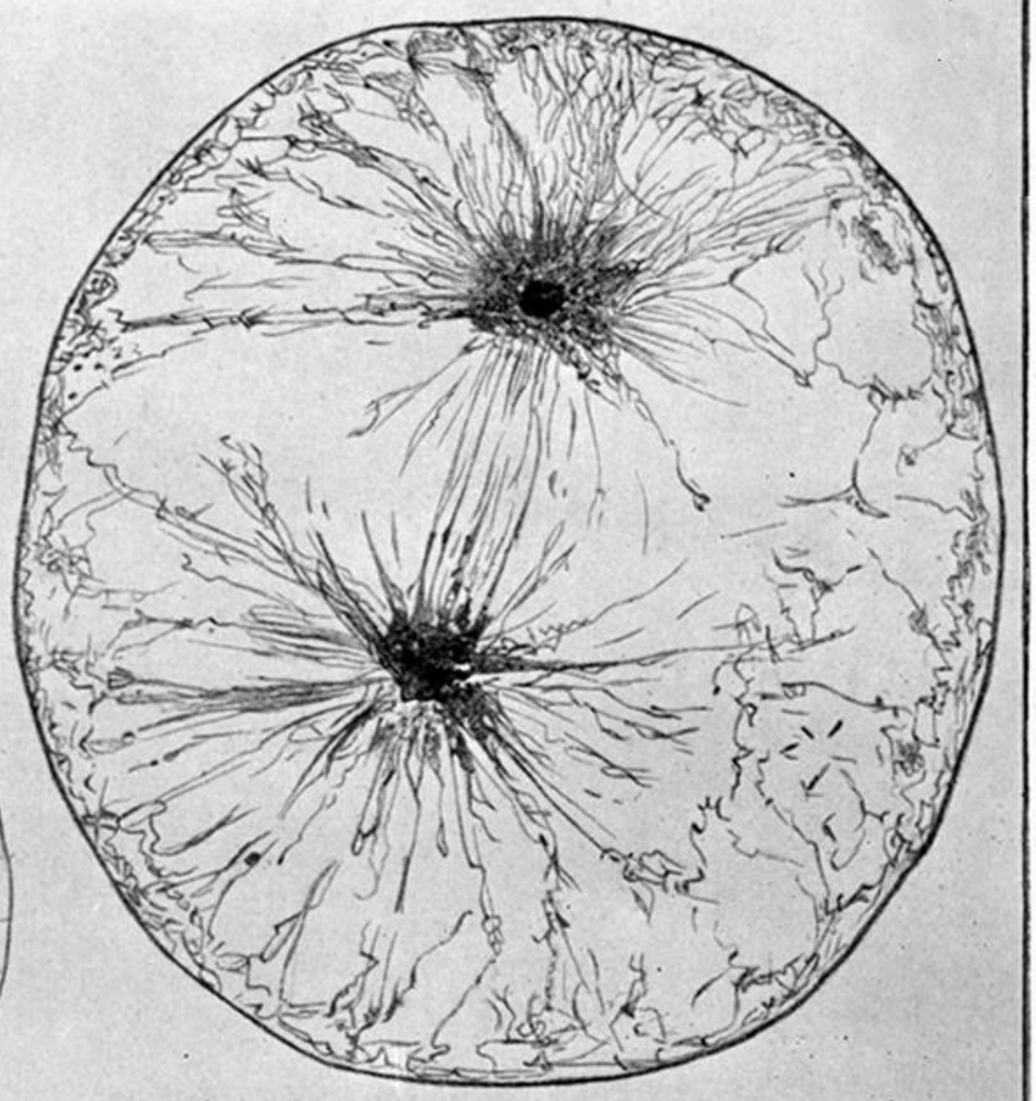
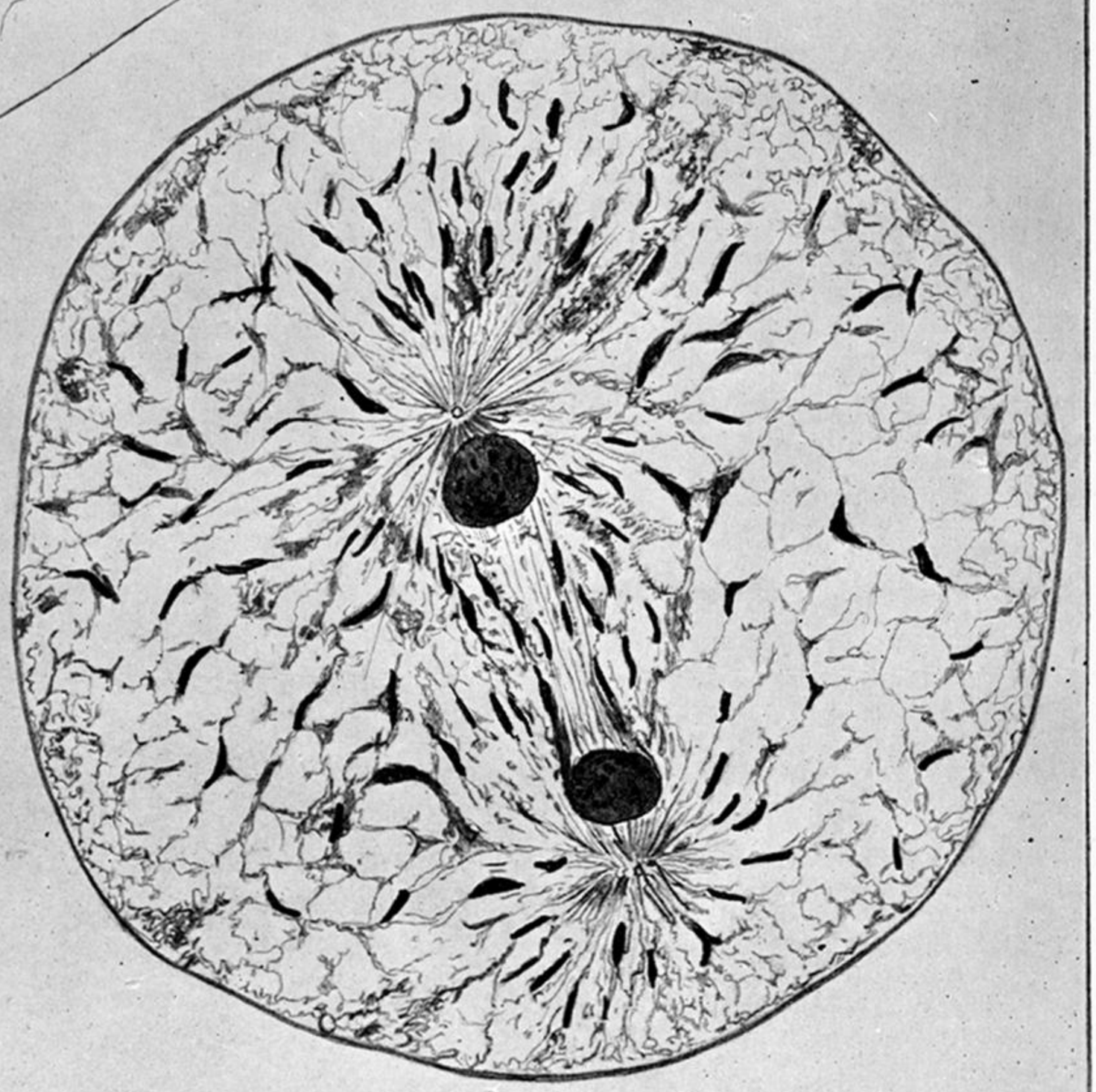


Fig. 34.



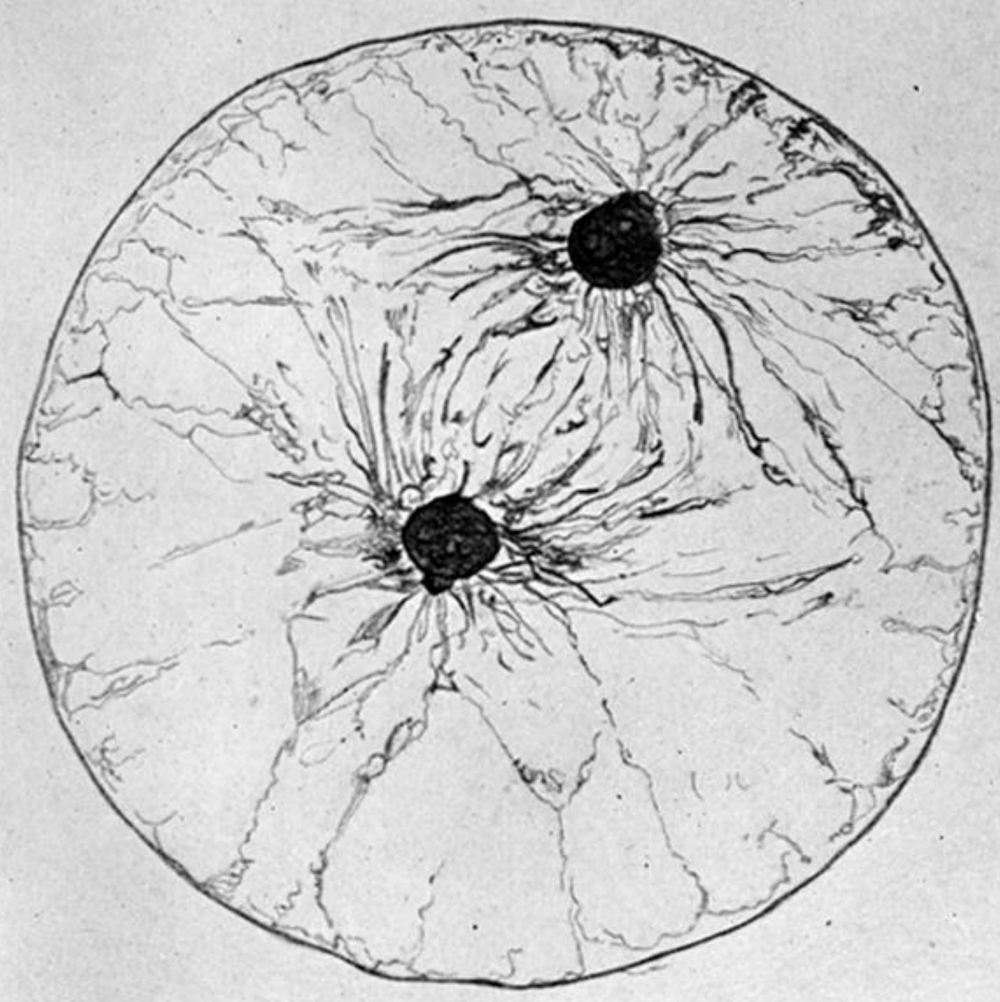


Fig. 35.

Fig. 33.

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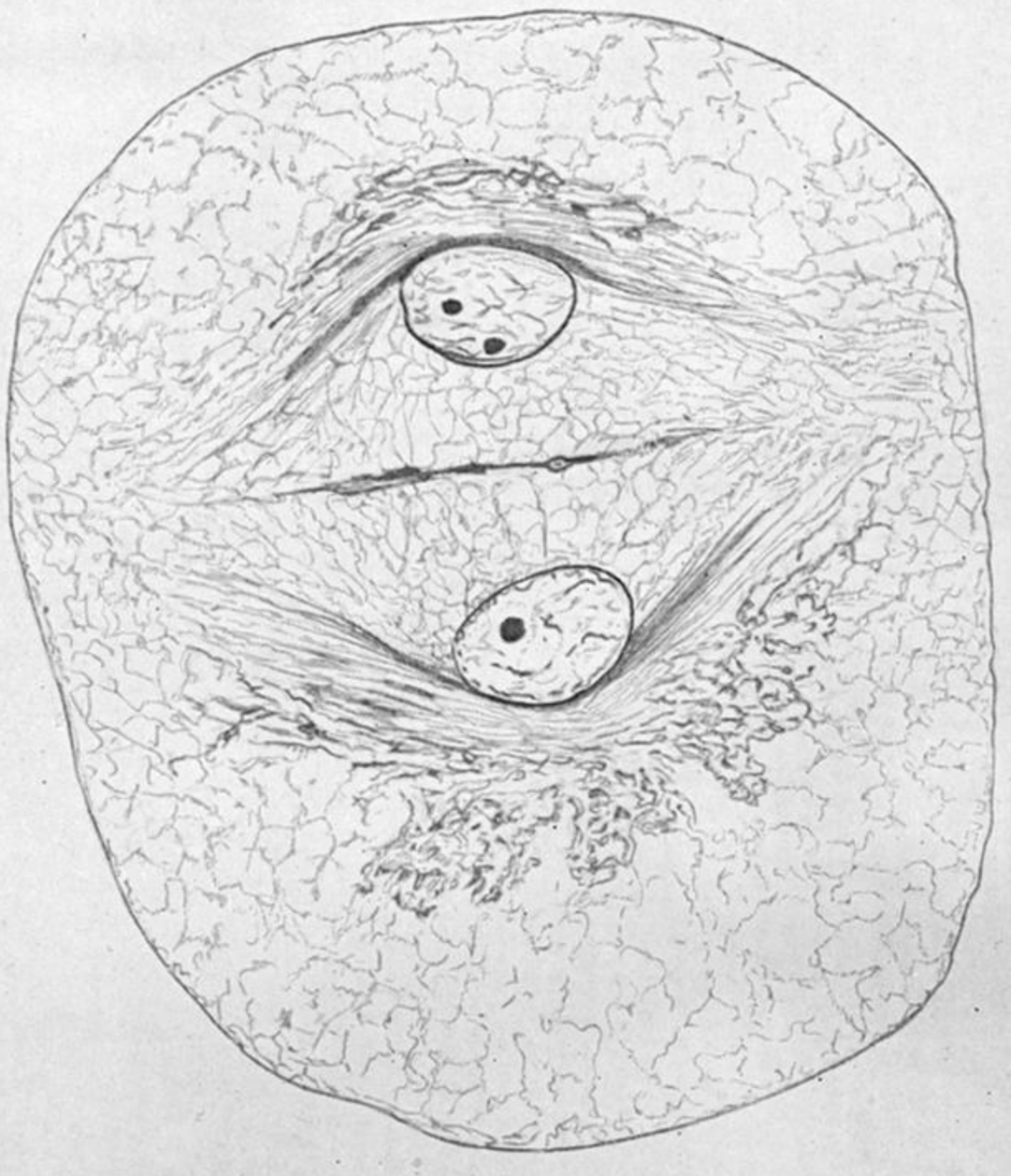
PLATE 22.

Figs. 34, 35. *Ascophyllum.* End of first nuclear division in germinating spore.



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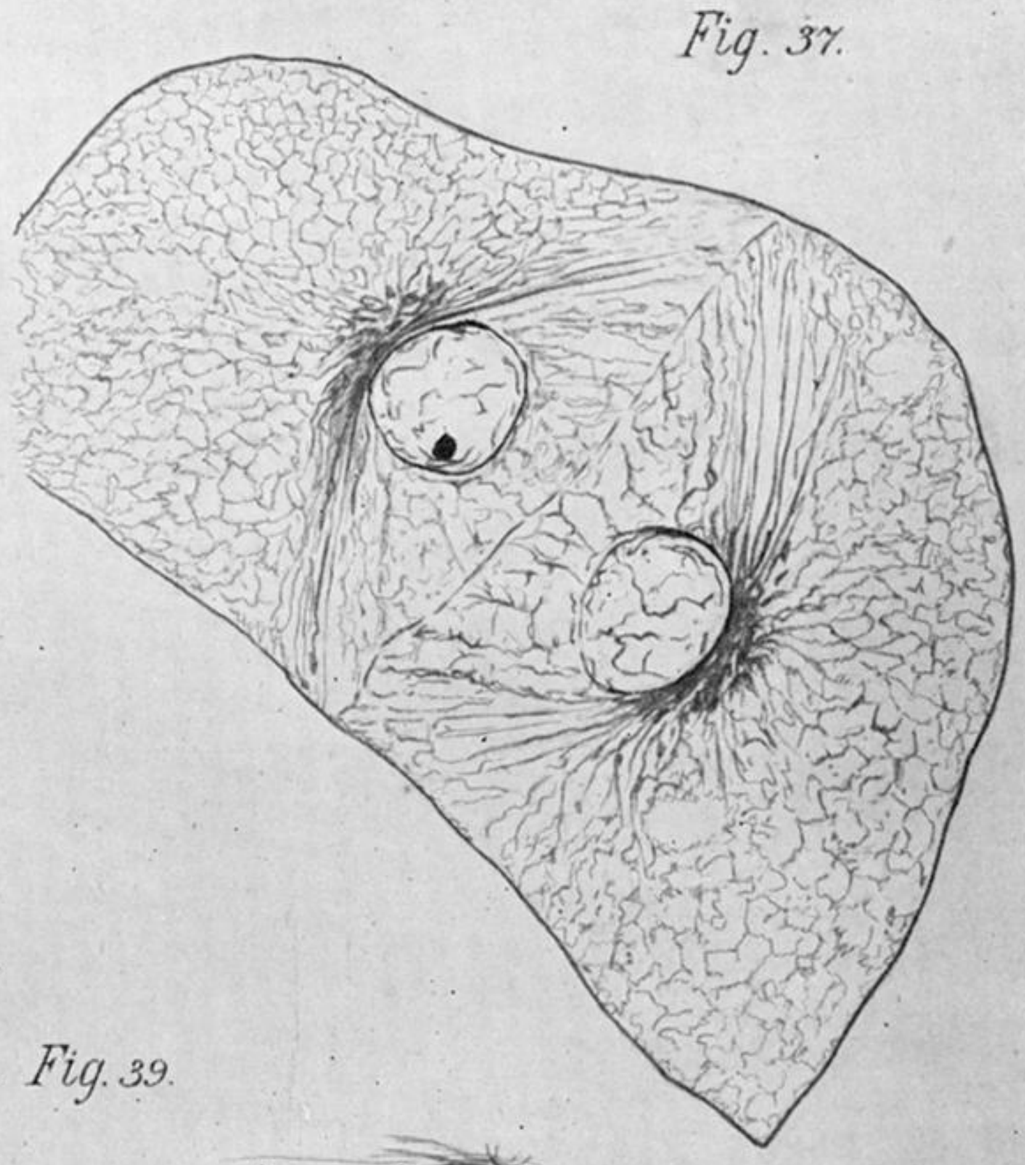

Fig. 37.

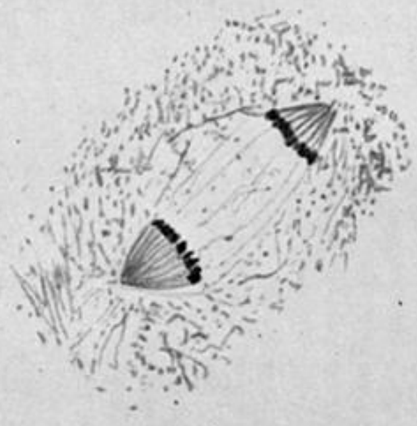

Fig. 38.

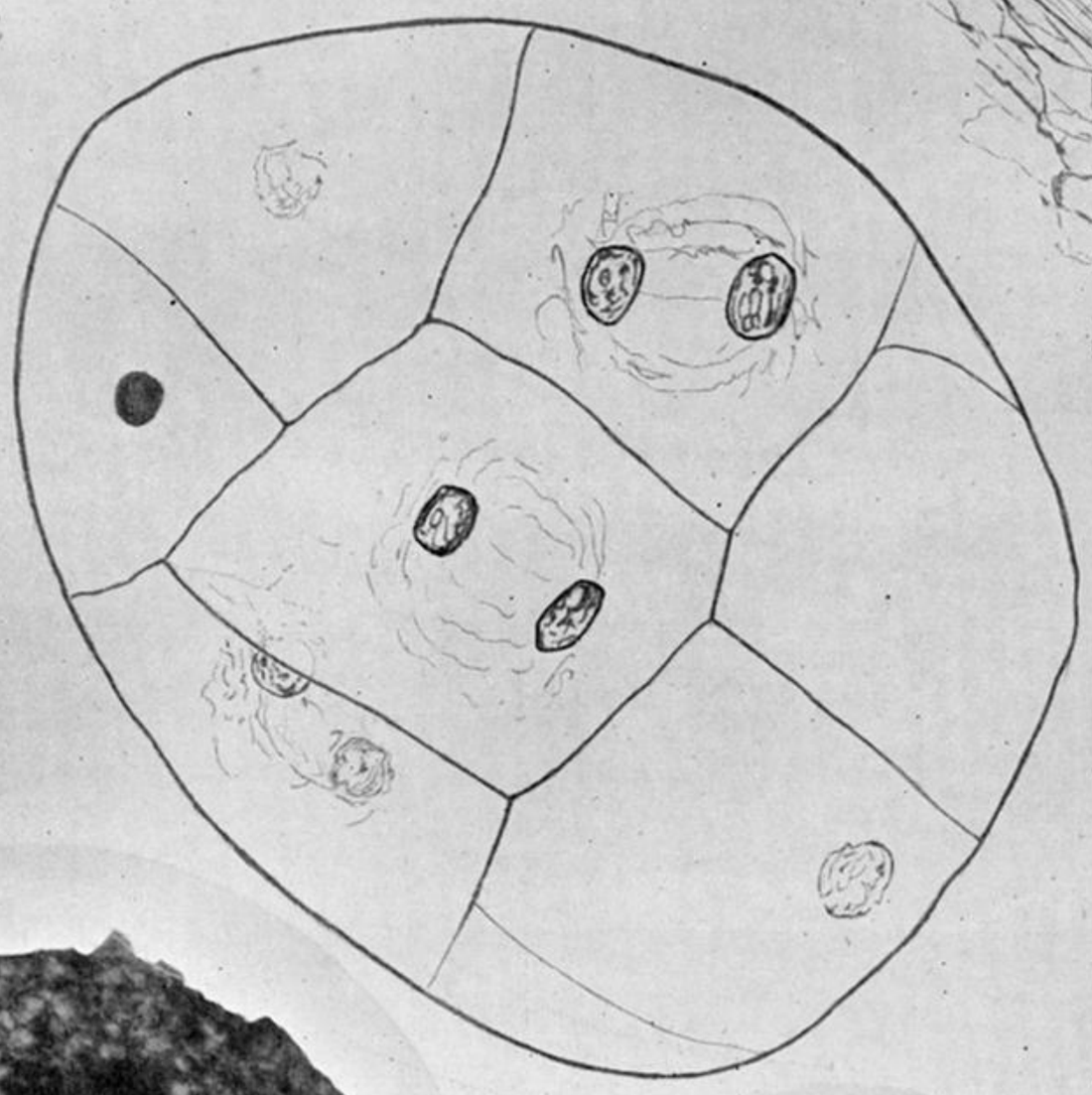

Fig. 40.

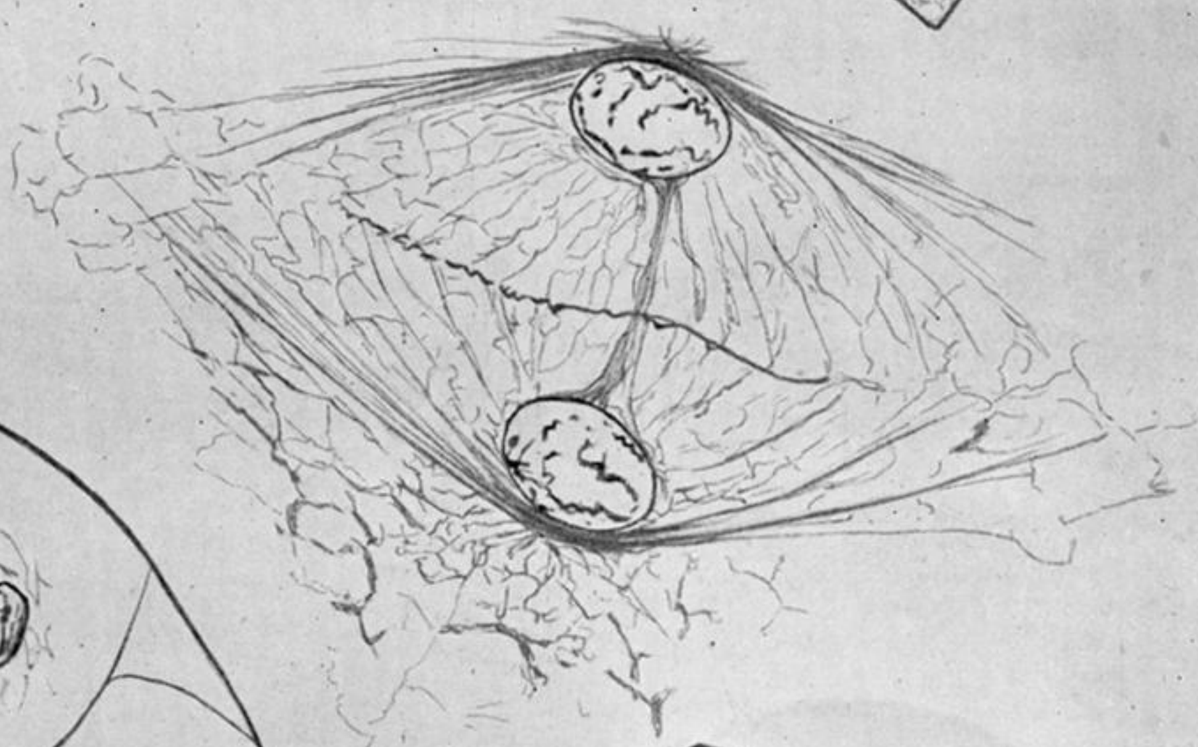

Fig. 39.

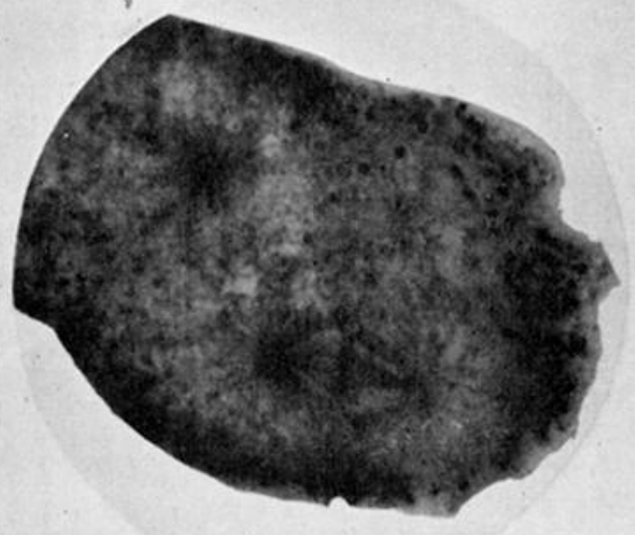

Fig. 42.

Fig. 41.

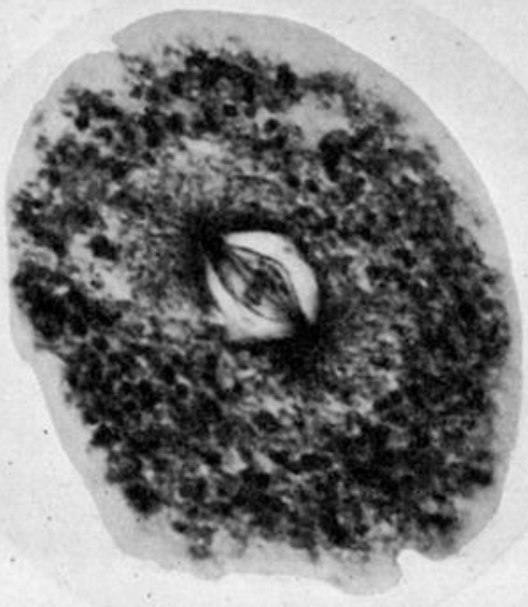

Fig. 43.

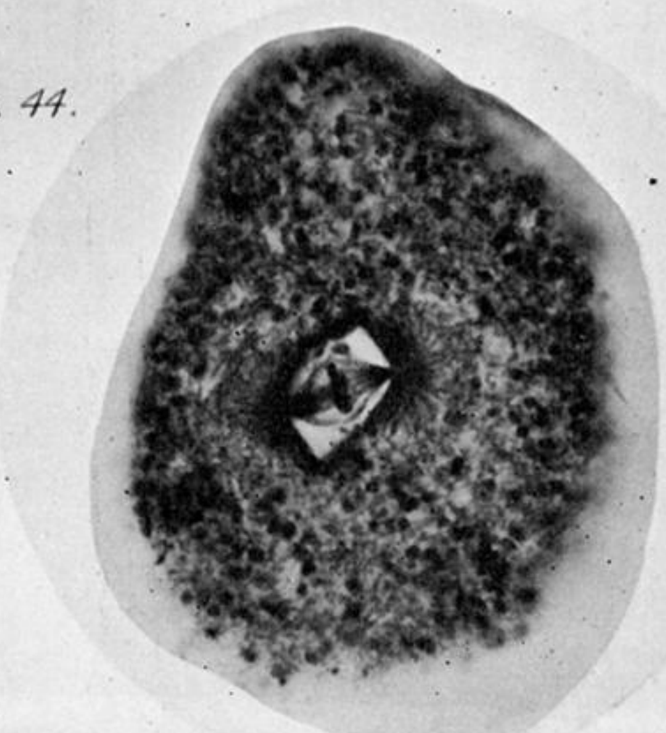

Fig. 44.

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PLATE 23.

Fig. 36. *Pelvetia canaliculata.* First nuclear division in the spore. *c*, the cell plate.
Figs. 37, 38. Second division in preceding plant.
Fig. 39. Third division in same.
Fig. 40. Multicellular embryo of *Ascophyllum.*

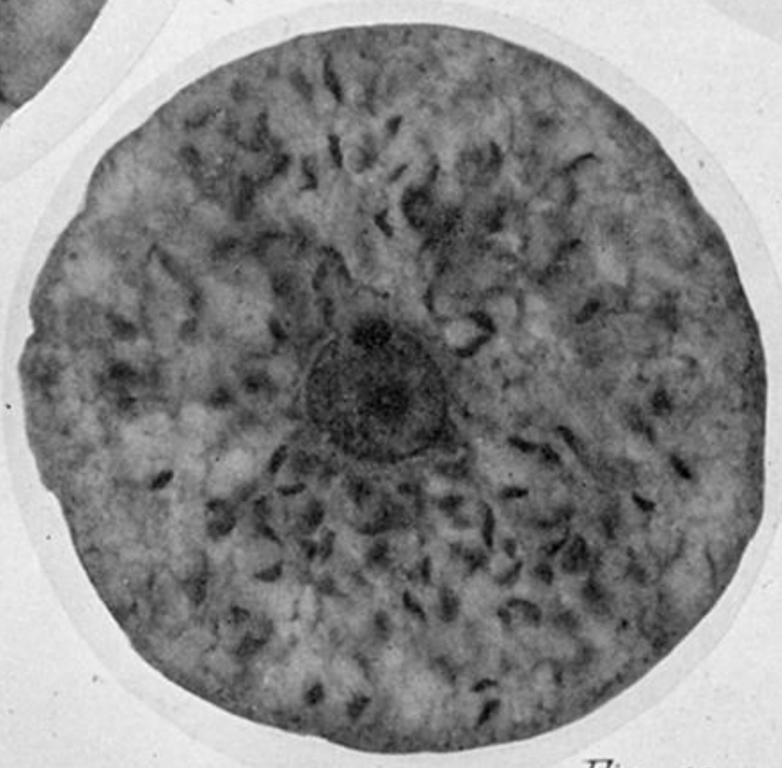
Photomicrographs.

All (except fig. 45) magnified about 1,000 diameters.

Figs. 41–44. Stages in the last nuclear division of the nucleus in the oogonium of *Fucus vesiculosus.*

Fig. 46.

Fig. 45.



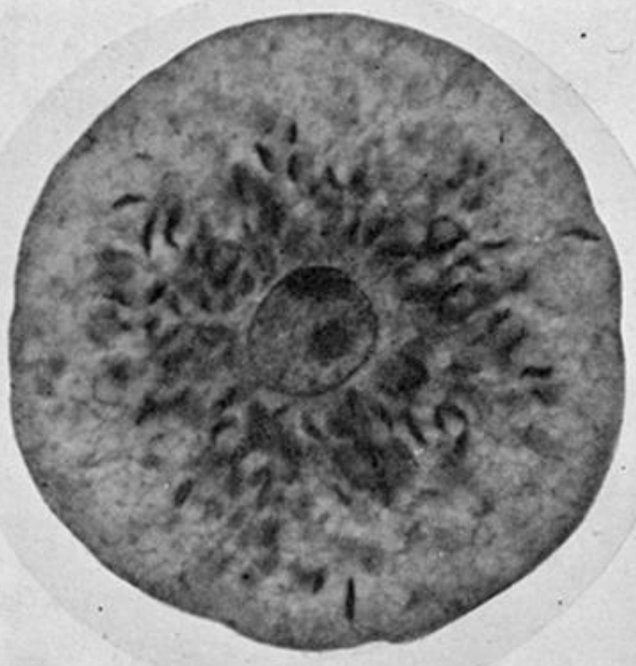


Fig. 49.

Fig. 48.

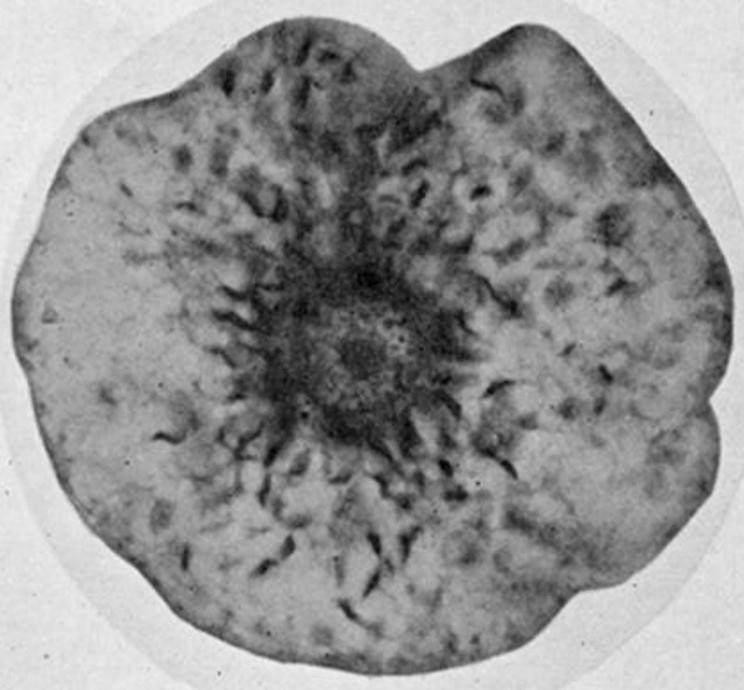


Fig. 47.

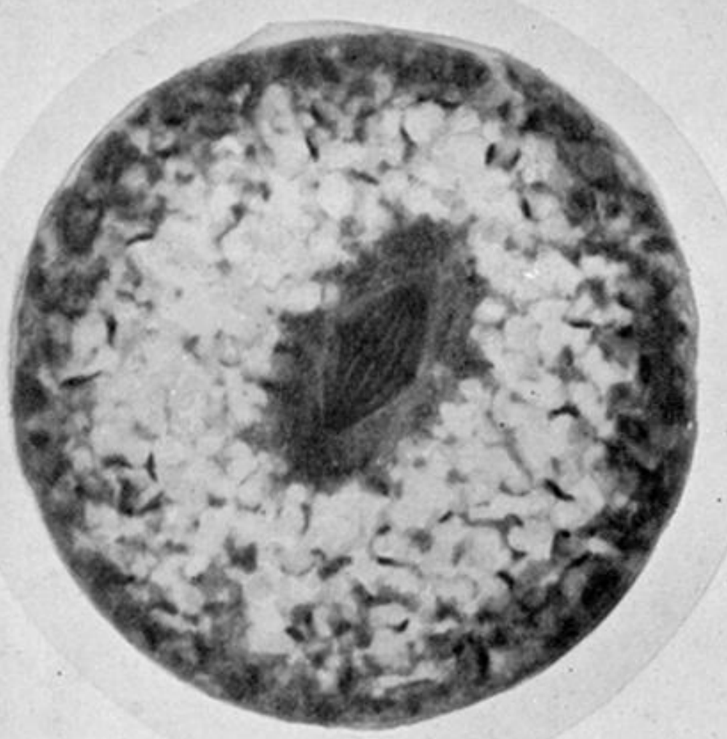


Fig. 50.

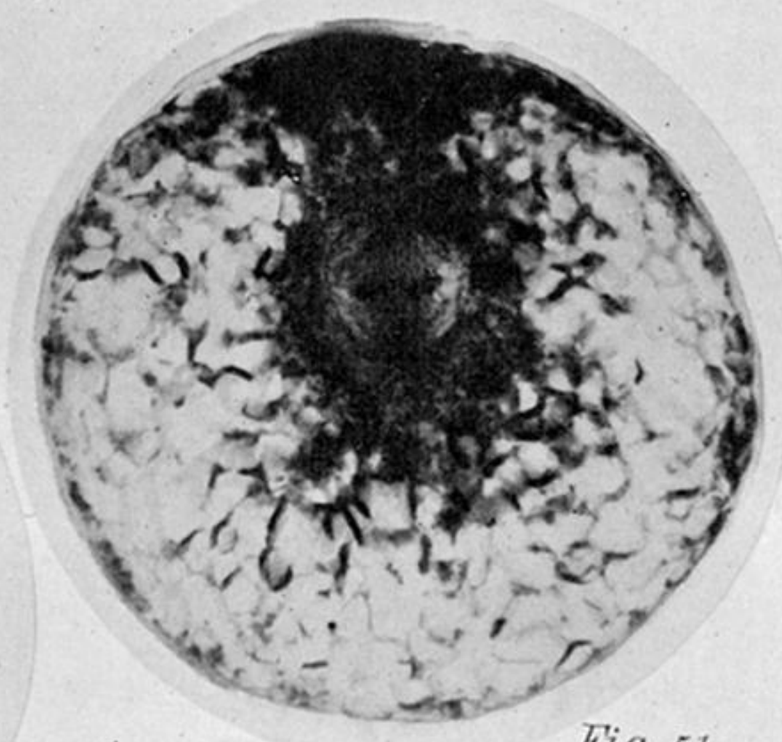


Fig. 51.

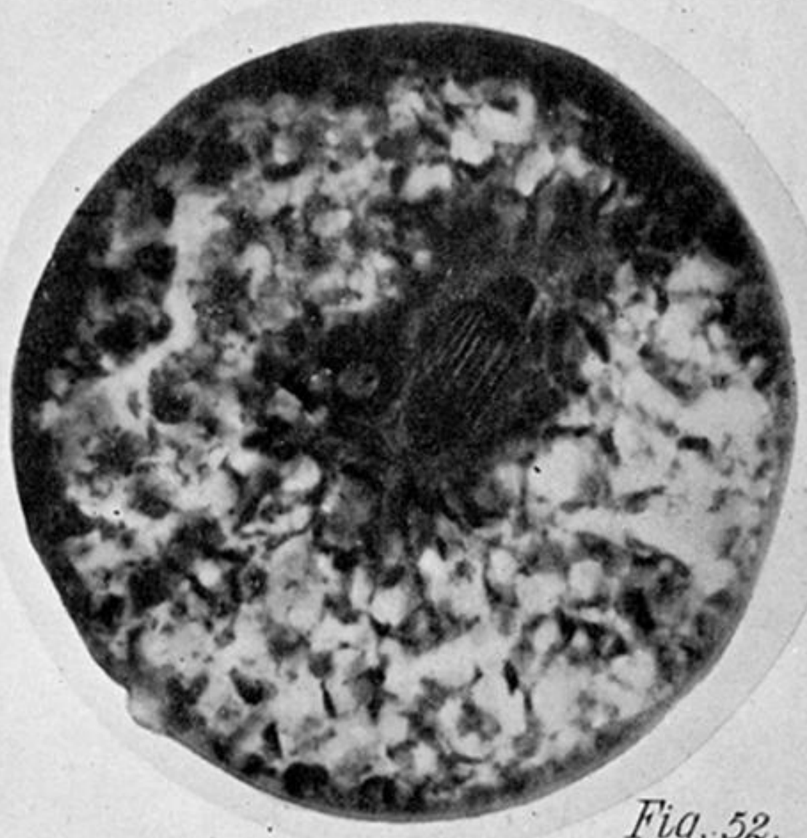


Fig. 52.

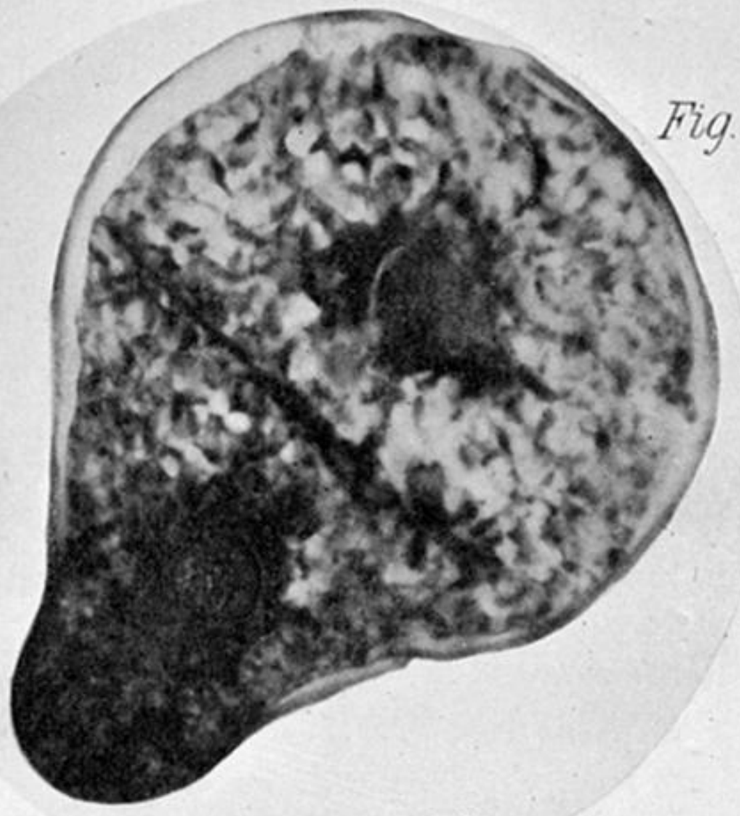


Fig. 55.

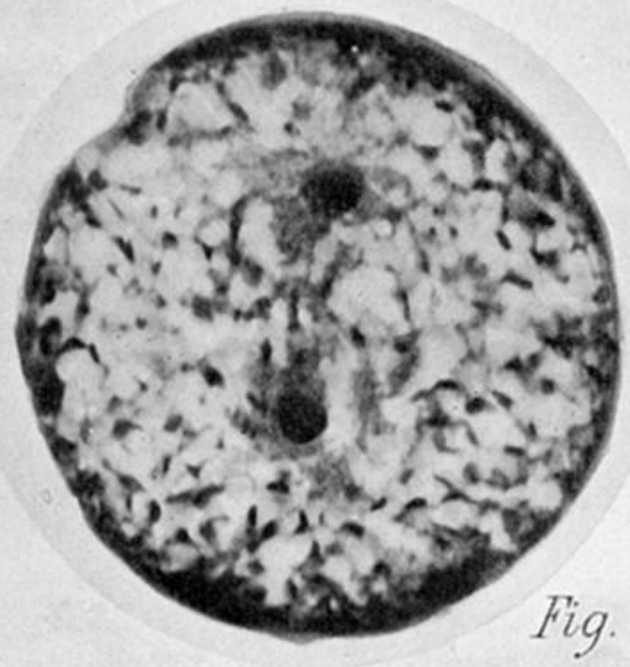


Fig. 54.

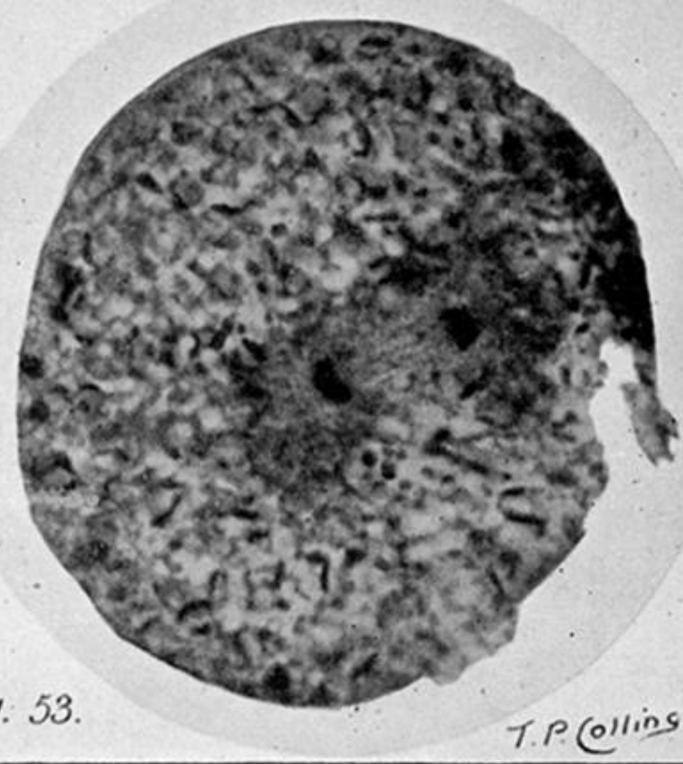


Fig. 53.

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PLATE 24.

Fig. 45. Divided oogonium of *F. vesiculosus*, one oosphere containing two nuclei. (× 600.)

Fig. 46. Portion of oogonium extruded amongst mature eggs, showing two nuclei in focus.

Figs. 47–49. Stages in fertilisation of *Ascophyllum nodosum*.

Figs. 50–54. *Fucus vesiculosus*. First nuclear division in the oospore.

Fig. 55. *F. vesiculosus*. Oospore with rhizoid and two nuclei.